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13. ABSTRACT (Maximum 200 Words)

Prostate cancer skeletal metastases are considered osteoblastic; however, histopathological examination usually reveals underlying osteoclastic activity. A key molecule required for induction of osteoclastic activity is receptor activator of NFkB ligand (RANKL). RANKL activity is opposed by osteoprotegerin (OPG). Thus, the balance of RANKL and OPG in the prostate cancer tissue may regulate the overall phenotype of the metastatic lesion. We demonstrated that inhibition of RANKL activity diminishes progression of initiation and progression of skeletal metastases. We also identified that the RANKL promoter is active in bone and induced by transforming growth factor-beta (TGF-b). Taken together, these data indicate that RANKL is a sound molecular target for prostate cancer bone metastases.

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INTRODUCTION: Prostate cancer skeletal metastases are considered osteoblastic; however, histopathological examination usually reveals underlying osteoclastic activity (reviewed in 1). A key molecule required for induction of osteoclastic activity is receptor activator of NFkB ligand (RANKL). RANKL activity is opposed by osteoprotegerin (OPG). Thus, the balance of RANKL and OPG in the prostate cancer tissue may regulate the overall phenotype of the metastatic lesion. We have determined that prostate cancer cells express increasing levels of RANKL and decreasing levels of OPG. Additionally, we have determined that androgen promotes OPG expression at the transcriptional level. Thus, loss of androgen may reduce OPG expression and favor a shift towards RANKL activity. Additionally, in a murine model, we have demonstrated the ability to inhibit establishment of prostate cancer in bone by blocking RANKL-induced osteoclastic activity using OPG. However, OPG can bind pro-apoptotic molecules and block apoptosis of cancer cells indicating that it may not be useful for clinical use (2). Instead, alternative methods to block RANKL activity may be more clinically relevant. Based on our previous findings and those of others our hypothesis is that an increase in the RANKL:OPG ratio contributes to the development of CaP skeletal metastases. Accordingly, a corollary hypothesis is that restoring the RANKL:OPG axis through inhibition of RANKL activity will diminish progression of skeletal metastases. Accordingly, the specific aims of this project are to (1) identify the mechanisms through which OPG expression is regulated in CaP cells and (2) determine if inhibition of RANKL activity by methods other than OPG can block the establishment and progression of CaP skeletal metastases in vivo.

BODY: Original Tasks:

Task 1. Identify the mechanisms through which OPG expression are regulated in CaP cells (Months 1-24):

a. Determine OPG promoter activity in other CaP cells (Months 1-6).
    i. Transfect cells with OPG promoter reporter and treat with dihydrotestosterone.
b. Define cis-acting sites that are responsible for activation and androgen response of the OPG promoter in CaP cells (Months 7-24).
    i. Transfect cells with serially deleted OPG promoter-reporter vectors (Months 7-10).
    ii. Create and characterize activity of 50 bp deletion mutants based on information from Task 1bi (Months 11-14).
    iii. Clone into reporter vector and characterize activity of 50 bp fragment (from Task 1bii) (Months 15-19).
    iv. Create and characterize activity of point-mutated 50 bp fragment (Months 20-24).

Task 2. Determine if inhibition of RANKL activity by methods other than OPG can block the establishment and progression of CaP skeletal metastases in vivo (Months 12-36).

a. Evaluate effect of sRANK on prostate cancer establishment in bone (Months 12-17)
b. Evaluate effect of sRANK on prostate cancer progression in bone (Months 18-24)
c. Determine effect of anti-RANKL antibody on prostate cancer establishment in bone (Months 25-30)

d. Determine effect of anti-RANKL antibody on prostate cancer progression in bone (Months 31-36).

Task 1 a i was completed in months 1-6 and was reported in FY1 annual report.
Task 1 b i was completed in months 7-12 and was reported in FY1 annual report.
In summary, those results showed that using five different lengths of the OPG promoter that dihydrotestone (DHT) at 50 nM generally inhibits the longer length OPG promoters, but at 100 nM it induces these OPG promoter in C4-2B prostate cancer cells (previous report). This resulted in not providing clear and unequivocal evidence for the location of an androgen responsive element in the OPG promoter. Without this information, it would not be possible to continue the proposal as outlined. Thus, in FY2 (the subject of this report) we chose to better identify the location of an androgen responsive component of the OPG promoter prior to pursuing shorter (50 bp) deletions of the OPG promoter (Task 1 b ii) and the subsequent Tasks (1 b iii and 1 b iv).

In FY2 we did the following:
The observation in FY1 work that higher levels of DHT inhibited OPG promoter activity was reminiscent of the biphasic response scene with PSA and DHT, which first induces PSA, then inhibits PSA production. To further explore if this biphasic response could be seen at more physiologic levels in the shorter promoter (OPG4), we did a dose response study at closer to physiological levels (0.1 to 10) with the OPG4 promoter. We found that DHT induced the OPG4 promoter in a dose responsive fashion. This suggested that the OPG4 contains a response element that is driven down by androgen (Figure 4). However, it could still be downstream of this fragment.

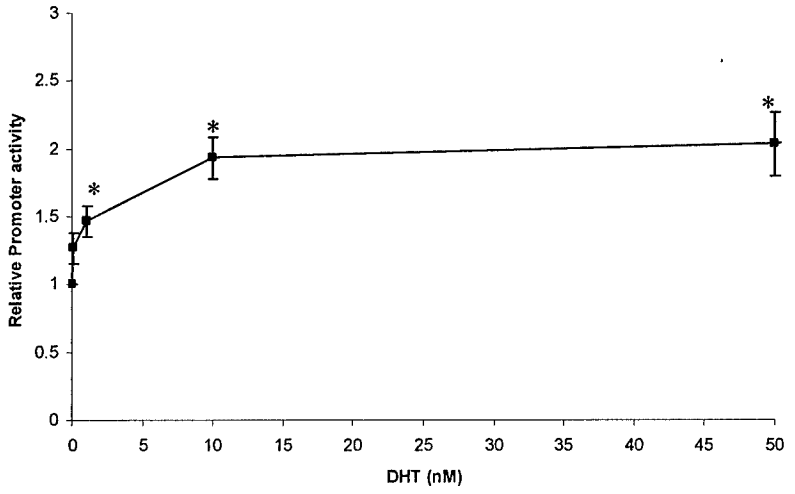


Fig. 1. DHT induces OPG promoter activation in a dose-respondent fashion. C4-2B prostate cancer cells were transfected with the OPG 4 promoter construct treated with the indicated level of DHT. Twenty-four hours later total cell lysate was collected for measurement of luciferase. *P<0.05 for the interaction of dosage and time; ANOVA and Fisher's protected least significant difference for post-hoc analysis.

To extend these studies and provide a better sense of the applicability of these findings to other prostate cancer cell lines, we evaluated DHT's ability to induce the different lengths of the OPG promoter in both the LNCaP can PC-3 cell line. PC-3 cells were stably transfected with the human androgen receptor prior to this study as they are androgen receptor negative. DHT at physiological levels (10 nM) induced the OPG promoter in both cell lines (Fig. 3). In contrast to that observed in C4-2B cells in the FY1 work, the longer promoters in both LNCaP and PC-3 were induced by DHT.

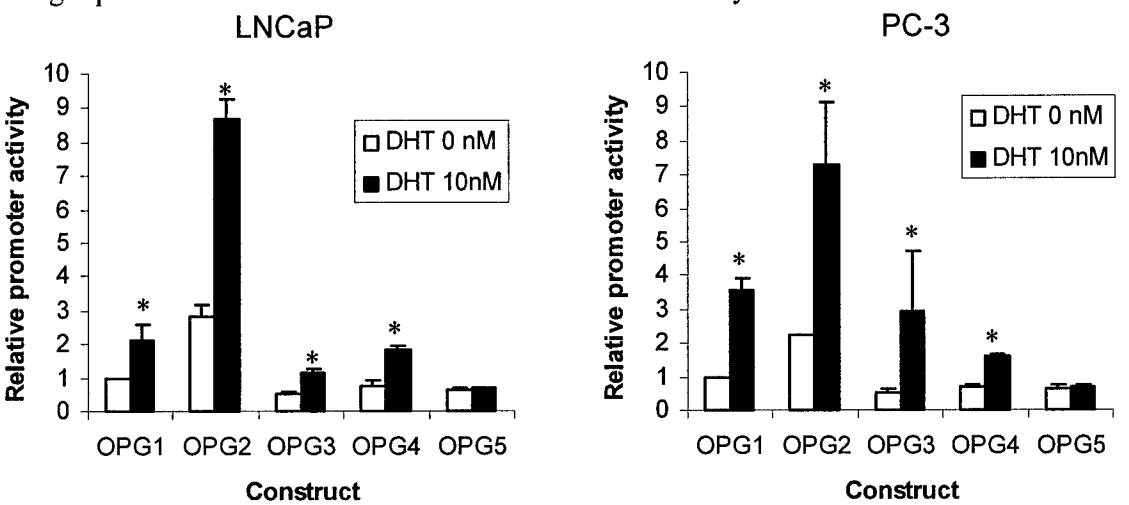
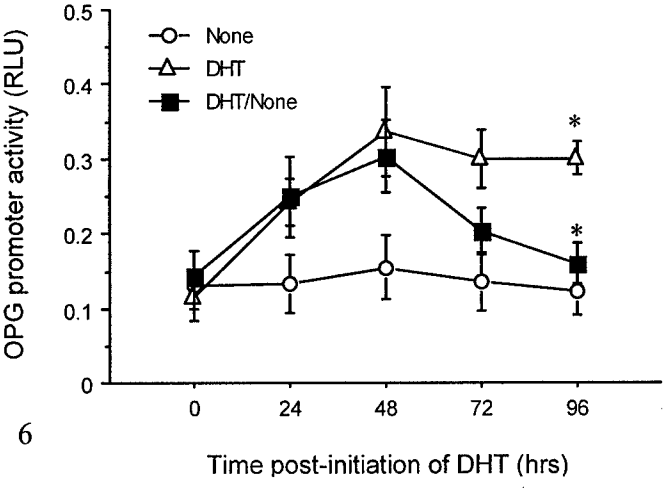


Figure 2. DHT induces OPG activity in prostate cancer cells. Prostate cancer cells were transfected with the OPG promoter constructs of different length and treated with the indicated level of DHT. Twenty-four hours later total cell lysate was collected for measurement of luciferase. *P<0.05 versus DHT 0 nM for each respective construct; ANOVA and Fisher's protected least significant difference for post-hoc analysis.

In vitro studies do not necessarily reflect in vivo events. To provide evidence that DHT effects the OPG promoter in vivo, we stably transfected the OPG4 promoter into C4-2B cells and implanted these cells subcutaneously into orchiectomized SCID mice. Each mouse was injected in 5 locations so that 5 tumors would develop. Tumors were allowed to become established until they reached approximately 100 mm^3. At that time, mice were injected with DHT (10 µl of a 40 µM solution intravenously via the tail vein) or PBS vehicle every 24 hours and tumors were collected at the indicated times. Within 24 hours, DHT induced OPG promoter activity, which peaked at 72 hours and maintained these levels for up to 96 hours, at which time the study was terminated (Fig. 3). In one group, DHT was administered for the first 48 hours, and then treatment was stopped. In those animals, the initial rise in DHT was observed and the OPG promoter activity declined by 96 hours.

Figure 3. DHT activates the OPG promoter in vivo. See text for description of experiment. Tumors were excised at indicated times post-initiation of DHT treatment, tumors were homogenized to obtain total protein lysate, which was normalized for protein content. The protein was then subjected to luciferase assay. *P<0.01 for time*treatment interaction. Repeated measures ANOVA.



In all of these studies, OPG 4 was induced by DHT, but OPG 5, which is deleted from OPG 4 downstream to OPG 5 is not induced by DHT. These findings now provide strong evidence that the fragment between OPG 4 and OPG 5 contains an androgen responsive element. Thus, the completion of all these studies in FY 2 (months 12-24) provides strong evidence that we have identified the appropriate region of the OPG promoter to target for further mutational analysis. However, our DOD project was funded for only two years as opposed to three, thus we will continue to perform this component of the study through other resources. In essence, we did not accomplish Tasks 1 b ii through 1 b iii in FY2 in order to ensure we identified the appropriate region of the OPG promoter for mutational analysis.

Task 2:

Task 2a and b which were supposed to be performed in FY2 months 12-24; were actually completed early and reported in FY1 report and published at that time. This work is presented in the Cancer Research publication attached to the FY1 report. Additionally, we had extended these studies to delineate how RANKL expression is regulated in bone. We identified that the RANKL promoter is activated in bone by transforming growth factor-beta. This work was presented in the Prostate publication attached to the FY1 report. We were not funded for FY 3, but since we completed FY2 early in FY1, we set out to pursue the next component. Thus, the next component was to perform Tasks 2 c and 2 d. The original intent of these tasks was to repeat the FY2 experiments using an antibody to RANKL; however, this antibody become unavailable to us (was supposed to be supplied by Amgen and they could not provide it) and we could not complete Task 2 c and 2 d. However, in FY2 we continued to explore the in vivo ability of inhibiting RANKL activation on prostate cancer cell growth in bone. Our goal is to determine if sRANK-Fc could act as an adjuvant to docetaxel chemotherapy of prostate cancer in bone.

To attack this goal, our first task was to determine the minimum and maximum effective doses of docetaxel on prostate cancer cells. Accordingly, mice were injected subcutaneously and within the tibia with C4-2B cells and docetaxel or vehicle treatment was initiated. Tumor volumes were measured weekly and radiographs of the tibia were taken at the end of the study. Docetaxel at all doses effectively decreased subcutaneous tumor growth (Fig. 4) and decreased establishment of tumors in the tibia (Table 1) and (Fig. 5).

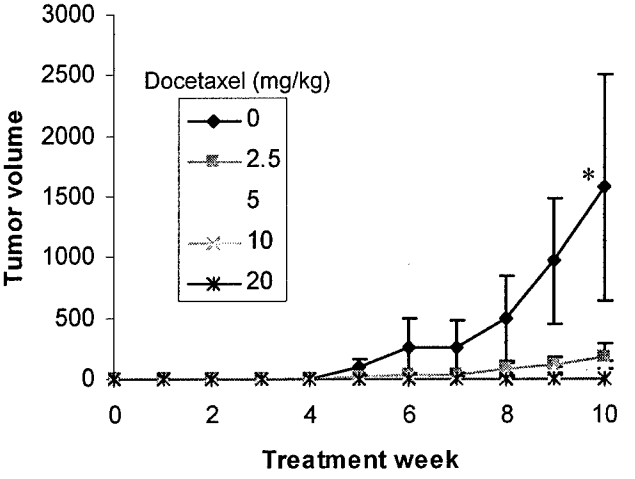
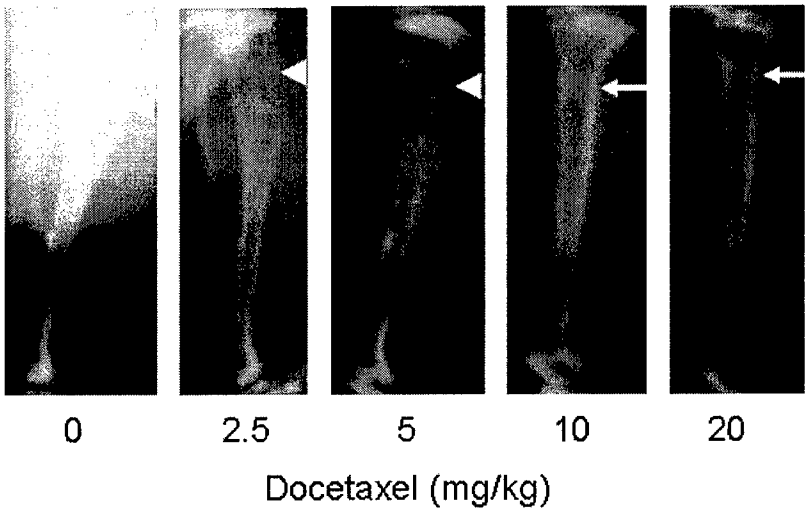


Figure 4. Docetaxel inhibits establishment of C4-2B subcutaneous tumors. Data are shown as mean±SEM. Repeated measures ANOVA ($p=0.03$) and *$P<0.01$ vs. all docetaxel treatments. (Fisher's protected least significant difference).

Table 1. scored radiographically based on the a

	Docetaxel (mg/kg)				
Tumor score	0	2.5	5	10	20
-	2	3	1	4	10
+	1	1	2	5	0
++	3	2	6	0	0
+++	2	3	0	0	0

Figure 5. Docetaxel decreases establishment of C4-2B tumors in bone. C4-2B cells were injected intratibially at which time docetaxel administration at the indicated dose was initiated. After 10 weeks, mice were sacrificed and tibiae radiographed. Arrowhead indicates osteolytic area and loss of cortex. Arrow indicates no boney changes (note intact cortex).



These experiments were repeated using the LuCaP 35 human prostate cancer xenograft model. The results were similar to the above experiment with C4-2B cells. Briefly, Docetaxel decreased tumor growth at subcutaneous sites (data not shown) and inhibited establishment of tumor in the tibia (Table 2).

Table 2. Effect of docetaxel on establishment of LuCaP 35 intratibial tumors. Tumors were scored radiographically based on the area of bone affected by tumor.

	Docetaxel (mg/kg)				
Tumor score	0	2.5	5	10	20
-	1	1	5	7	8
+	1	4	3	1	2
++	4	1	0	2	0
+++	1	2	2	0	0

Taken together, these data indicate that docetaxel is highly effective at blocking establishment of prostate cancer in bone. Also, they indicate that in order to test inhibition of RANKL using sRANK-Fc as an adjuvant to docetaxel, we should use 5 mg/kg, which has some efficacy, but is suboptimal and will thus allow us to determine if sRANK-Fc has an additive or synergistic effect with docetaxel. These experiments are currently ongoing, but are not completed in FY2 as the dose experiments were performed in FY2.

KEY RESEARCH ACCOMPLISHMENTS:

FY1

- Demonstration that DHT regulates OPG in a biphasic fashion.
- Demonstration that sRANK-Fc can inhibition prostate cancer growth in bone but not soft tissue.
- Demonstration of RANKL gene promoter activity in vivo.
- Demonstrate that TGF-beta, a factor produce upon resorption of bone, induces the RANKL promoter
- Demonstrate that tumor volume measured in vivo by bioluminescence imaging correlates with tumor volume measured by PSA.

FY2

- Identified that the OPG promoter segment between OPG 4 and OPG 5 contains an androgen response element, both in vitro and in vivo.
- Demonstrated that docetaxel is highly effective against the establishment of intratibial prostate cancer growth
- Demonstrated that docetaxel prevents subcutaneous tumor growth
- Identified a suboptimal, but effective dose of docetaxel to use in combination with sRANK-Fc.

REPORTABLE OUTCOMES:

FY1

1. Zhang J, Dai J, Yao Z, Lu Y, Dougall W, Keller ET. Soluble RANK-Fc diminishes prostate cancer progression in bone. Cancer Res. 63:7883-7890, 2003.
2. Zhang J, Lu Y, Kitazawa R, Kitazawa S, Dai J, Zhao X, Yao Z, Pienta KJ, Keller ET. Role of TGF-β in Prostate Cancer Skeletal Metastases: In Vivo Real-time Imaging of TGF-β-induced RANK Ligand Transcriptional Activation in Prostate Cancer. Prostate. 59:360-369, 2004.

FY2

3. Keller ET. Mechanisms of bone resorption in prostate cancer skeletal metastases. In: F. Columbus, ed. Progress in Prostate Cancer Research. Nova Publishers. 2004.
4. Keller ET, Brown J. Prostate cancer bone metastases promote both osteolytic and osteoblastic activity. J Cell Biochem. 91:718-729, 2004.

CONCLUSIONS:

RANKL promotes prostate cancer growth in bone. Blocking RANKL is an effective strategy to diminish progression of prostate cancer growth in bone (3). Most likely it works through inhibiting osteoclastogenesis as the inhibitory effect is specific to tumor growing in bone as opposed to subcutaneous tumor.

Additionally, the bone environment may promote RANKL expression from tumor cells through release of factors that increase RANKL expression such as TGF-beta (4). This suggests there is a vicious cycle present that allows for increased bone resorption, release of prostate cancer-active growth factors, which in turn stimulates prostate cancer cells to

continue growth and effect bone remodeling (5). This may be tempered in the bone environment by production of OPG which is regulated by androgens. Specifically, it appears that DHT increases OPG expression through activation of the promoter. Thus, when men with advance prostate cancer are treated using androgen deprivation, then androgen levels will decline, which will result in decreased OPG allowing for unopposed RANKL activity and thus favor bone osteolysis (6).

REFERENCES:

(1) Logothetis, C. J. and Lin, S. H. Osteoblasts in prostate cancer metastasis to bone. Nat Rev Cancer, 5: 21-28, 2005.

(2) Holen, I., Croucher, P. I., Hamdy, F. C., and Eaton, C. L. Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells. Cancer Res, 62: 1619-1623, 2002.

(3) Zhang J, Dai J, Yao Z, Lu Y, Dougall W, Keller ET. Soluble RANK-Fc diminishes prostate cancer progression in bone. Cancer Res. 63:7883-7890, 2003.

(4) Zhang J, Lu Y, Kitazawa R, Kitazawa S, Dai J, Zhao X, Yao Z, Pienta KJ, Keller ET. Role of TGF-β in Prostate Cancer Skeletal Metastases: In Vivo Real-time Imaging of TGF-β-induced RANK Ligand Transcriptional Activation in Prostate Cancer. Prostate. 59:360-369, 2004.

(5) Keller ET, Brown J. Prostate cancer bone metastases promote both osteolytic and osteoblastic activity. J Cell Biochem. 91:718-729, 2004.

(6) Theoleyre, SY, Wittrant, Y, Tat, SK, FortunY, Redini F, Heymann, D. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev 15: 457-75, 2004.

APPENDICES:

Keller ET. Mechanisms of bone resorption in prostate cancer skeletal metastases. In: F. Columbus, ed. Progress in Prostate Cancer Research. Nova Publishers. 2004.

Keller ET, Brown J. Prostate cancer bone metastases promote both osteolytic and osteoblastic activity. J Cell Biochem. 91:718-729, 2004.

Chapter V

Mechanisms of Bone Resorption in Prostate Cancer Skeletal Metastases

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Abstract

Prostate cancer (CaP) frequently metastasizes to bone resulting in osteoblastic lesions with underlying osteoclast-mediated bone resorption. Skeletal metastases are often associated with significant complications including severe bone pain, impaired mobility, pathological fracture, and spinal cord compression and therefore demand advanced therapeutic interventions. Current therapeutic approaches for treatment of CaP include hormonal therapy, pharmacological management of bone pain, radiotherapy for pain and spinal cord compression, various chemotherapy regimens, and the use of bisphosphonates to inhibit increased osteoclast activity in bone metastases; however, there have only been limited advances in preventing or diminishing these bone lesions. Progress in defining osteoclast biology has led towards defining putative therapeutic targets to attack tumor-induced osteolysis.

Several factors have been found to be important in tumor-induced osteoclast activity and thus may serve as therapeutic targets. These include receptor activator of nuclear factor kappa B ligand, parathyroid hormone-related protein, interleukin-6, matrix metalloproteinases, endothelin-1 (ET-1), and cathepsin K (cat K). In this chapter, we review the roles of these factors in prostate cancer metastasis to bone and therapeutic methods to target these factors.

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Introduction

As prostate cancer progress, it typically metastasizes to bone. In addition to inducing osteoblastic activity (i.e., induce mineralization in the skeletal metastatic site), prostate skeletal metastases also have an underlying osteoclastic component. The prostate cancer-induced bone resorption causes pain and pathologic bone fractures. Continuing advances on osteoclast biology provide clues to understanding how osteoclasts contribute to tumor-mediated bone resorption. Due to the importance of osteoclast activity in skeletal metastases, there is a lot of research efforts toward defining clinical inhibitors of osteoclast activity. In this review, we will summarize the biology of osteoclasts and pro-osteoclastic factors produced by prostate cancer.

Osteoclast Biology

Osteoclasts are derived from the colony-forming unit granulocyte-macrophage (CFU-GM) hematopoietic precursor cells. The CFU-GM undergoes a defined progression of maturation steps that ultimately result in fusion of the precursor cells into mature osteoclasts (Fig. 1). Several factors promote osteoclastogenesis including growth factors and cytokines. Both colony stimulating factor (CSF-1) and interleukins-1 and -6 (IL-1 and IL-6) expand the osteoclast precursor pool. TNF-alpha promotes conversion of the promonocyte to a committed osteoclast precursor [1].

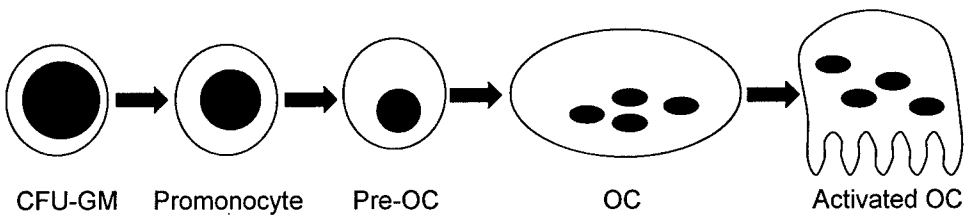

Figure 1. Cellular pathway for osteoclastogenesis. CFU-GM, colony forming unit-granulocyte-macrophage; OC, osteoclast.

Although several factors promote osteoclastogenesis, one factor that is required for production of mature osteoclasts is receptor activator of nuclear factor kappa B ligand (RANKL). A member of the tumor necrosis factor family, RANKL is initially expressed by bone marrow stromal cells, osteoblasts, and activated-T cells. RANKL is most commonly a membrane anchored molecule; however, a small fraction of RANKL is released through proteolytic cleavage from the cell surface as a soluble 245 amino acid homotrimeric molecule (sRANKL) [2]. Both soluble and membrane bound RANKL promote osteoclast formation and activation by binding to RANK on the osteoclast precursor membrane (Fig. 2) [2-6] that has the characteristics of a monocytes [7]. RANKL binding to RANK induces NFkappaB and Fos activation [8, 9]. Several lines of evidence demonstrate RANKL's importance in osteoclastogenesis. For example, RANKL has been shown to induce osteoclastogenesis in vitro from CFU-GM [10]. Mice that are genetically engineered to overexpress RANKL or

RANK are severely osteoporotic [11]. Additionally, mice that have had their RANKL [12] or RANK [13] gene deleted have no osteoclasts and are osteopetrotic.

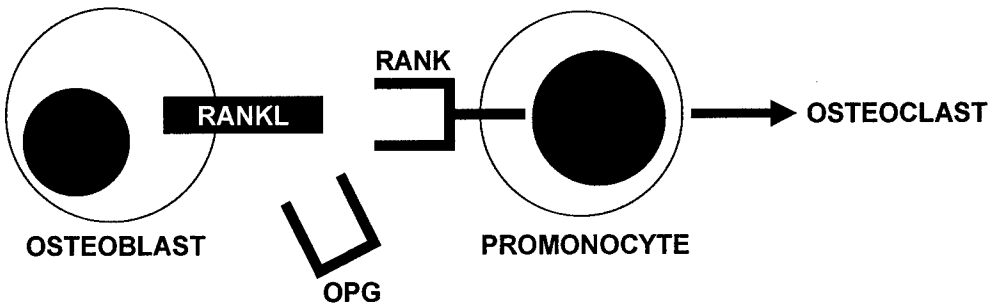

Figure 2. RANKL and OPG regulation of osteoclastogenesis.

In addition to RANKL and RANK, another key modulator of osteoclastogenesis is osteoprotegerin (OPG) (also known as osteoclastogenesis inhibitory factor-OCIF) [14, 15]. OPG serves as a decoy receptor that binds RANKL and thus blocks its ability to bind to RANK and induce osteoclastogenesis. In contrast to RANKL and RANK, whose expression is mainly restricted at low levels to the skeletal and immune systems, OPG is expressed in a variety of tissues, such as liver, lung, heart, kidney, stomach, intestines, skin, and calvaria in mice and lung, heart, kidney, and placenta in human [14, 16-21]. In bone, OPG is mainly produced by osteoblastic lineage cells and its expression increases as the cells become more differentiated [19, 22, 23]. Several factors including, 1,25-dihydroxyvitamin D3, IL-1-β, TNF-α, and BMP-2 induce OPG mRNA expression in human osteoblast cell lines [19]. Administration of recombinant OPG to normal rodents resulted in increased bone mass [14, 17] and completely prevented ovariectomy-induced bone loss without apparent adverse skeletal and extraskeletal side effects [14]. Additionally, a single subcutaneous injection of OPG is effective in rapidly and profoundly reducing bone turnover for a sustained period in women [24]. In fact, based on this activity, the balance ratio of RANKL to OPG appears to be very important in controlling the overall activity (i.e., lysis vs. no lysis) that will be observed [11, 23, 25, 26].

Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL)

As described above, RANKL is a key osteoclastogenic factor. Several lines of evidence support the role of RANKL in prostate cancer-mediated osteolysis. Although a bone metastatic prostate cancer cell line has been shown to express OPG [27], that same line overexpresses RANKL [28]. Additionally, in normal prostate, OPG protein was detected in luminal epithelial and stromal cells (5% to 65% and 15% to 70%, respectively) and RANKL immunoreactivity was observed in 15% to 50% of basal epithelial cells, 40% to 90% of luminal epithelial cells, and 70% to 100% of stromal cells [29]. OPG was not detected in 8 of 10 primary CaP specimens, but RANKL was heterogeneously expressed in 10 of 11 CaP

specimens [29]. Importantly, the percentage of tumor cells expressing OPG and RANKL was significantly increased in all CaP bone metastases compared with nonosseous metastases or primary CaP. Serum OPG levels are elevated in patients with advanced prostate cancer compared to less advanced prostate cancer [30]. However, RANKL levels were not measured in that study, thus one cannot determine if the ratio of RANKL:OPG was altered in these patients. It is possible that RANKL is only expressed locally at the skeletal metastatic site and therefore not detectable in the serum. Regardless, taken together, these observations suggest that the RANKL:OPG axis may play an important role in prostate cancer bone metastases. Further support for this possibility was demonstrated by the observation that administration of OPG prevented establishment of prostate cancer cells in the bones of SCID mice, although it had no effect on establishment of subcutaneous tumors in the same mice [28].

Parathyroid Hormone Related Protein (PTHrP)

PTHrP, a protein with limited homology to parathyroid hormone (PTH), was originally identified as a tumor-derived factor responsible for humoral hypercalcemia of malignancy (HHM). PTH and PTHrP bind to the same receptor (the PTH-1 receptor) and evoke the same biological activity due to similarities in their steric configurations at the region of 25-34 amino acids. Patients with solid tumors and hypercalcemia have increased serum PTHrP in 80% of the cases, emphasizing the impact of this peptide to increase bone resorption and renal tubular resorption of calcium [31]. Subsequent to its characterization in HHM, PTHrP was found to be produced by many normal tissues including, epithelium, lactating mammary gland, and cartilage where it has an autocrine, paracrine or intracrine role [31].

PTHrP is an attractive candidate for influencing prostate carcinoma growth. PTHrP is produced by normal prostate epithelial cells, from which prostate carcinoma arises and PTHrP is found in the seminal fluid [32, 33]. PTHrP has been immunohistochemically identified in prostate carcinoma tissue in patients with clinically localized disease [34], is found in higher levels in prostate intraepithelial neoplasia than in normal prostate epithelium, is found in higher levels in prostate carcinoma than in benign prostatic hyperplasia [35, 36], and is found in human metastatic lesions in bone [37]. However, in some studies, expression of PTHrP receptor in prostate cancer appears to be more consistent then expression of PTHrP itself [38]. Overexpression of ras oncogene in immortalized prostate epithelial cells has been shown to promote PTHrP expression [39]. This may account for the increased expression of PTHrP as the cells progress to a malignant phenotype.

There is evidence that PTHrP can regulate malignant tumor growth in an autocrine manner in human renal cell carcinoma [40], enhance breast cancer metastasis to bone [41, 42], and act as an autocrine growth factor for prostate carcinoma cells *in vitro* [32] although it does not effect proliferation of normal prostate cells [43]. Recent evidence indicates that expression of nuclear-targeted PTHrP can protect prostate and other cells from apoptosis [37, 44], bind RNA [45], and act as a mitogen [46, 47]. PTHrP production by primary prostatic tumors is associated with increased tumor size and rate of growth in an animal model [37] suggesting that PTHrP acts in an autocrine or intracrine mechanism to promote tumor growth.

In contrast, in this same model and in an intracardiac injection model of prostate carcinoma, PTHrP was not associated with an increase in metastatic potential [37, 48]. This suggests that PTHrP is not important in the process of metastasis to bone but once in the bone microenvironment where target cells with receptors are present (osteoblasts), it may play a critical role in the bone response to prostate carcinoma. Of particular interest to prostate carcinoma, PSA has been shown to cleave PTHrP leading to an inactivation of the PTHrP-stimulation of cAMP which is a key pathway for the actions of PTHrP in bone [49]. Overexpression of PTHrP in prostate cancer cells has been shown to induce osteolytic lesions in the bone of rats [50] although the level of expression may not directly correlate with the degree of osteolysis [48]. All these data suggest that PTHrP has a critical role in the local bone microenvironment of metastatic prostate carcinoma; but what this precise role is has yet to be determined.

Interleukin-6 (IL-6)

IL-6 belongs to the "interleukin-6 type cytokine" family that also includes leukemia inhibitory factor, interleukin-11, ciliary neurotrophic factor, cardiotrophin-1 and oncostatin M [51]. Many physiologic functions are attributed to IL-6 including promotion of antibody production from B lymphocytes, modulation of hepatic acute phase reactant synthesis, promotion of osteoclastic-mediated bone resorption, and induction of thrombopoiesis [52]. IL-6 mediates its activity through the IL-6 receptor complex, which is composed of two components; an 80 Kd transmembrane receptor (IL-6Rp80, IL-6R, α-subunit) that specifically binds IL-6, but has no signaling capability and a 130 Kd membrane glycoprotein (gp130) that mediates signal transduction following IL-6R binding [53]. In addition to the transmembrane IL-6R, a soluble form of IL-6R (sIL-6R) exists that is produced by either proteolytic cleavage of the 80 kDa subunit [54, 55] or differential splicing of mRNA [56]. Although the sIL-6R does not posses a transmembrane component, it can still bind to IL-6 and the ligand bound sIL-6R·IL-6 complex activates signal transduction and biological responses through membrane-bound gp130 [57].

Multiple studies have demonstrated that IL-6 is elevated in the sera of patients with metastatic prostate cancer [58-60]. Adler et al. [58] demonstrated that serum levels of IL-6 and transforming growth factor-β1 are elevated in patients with metastatic prostate cancer, and that these levels correlate with tumor burden as assessed by serum PSA or clinically evident metastases. In a similar fashion, Drachenberg et al. [61] reported elevated serum IL-6 levels in men with hormone-refractory prostate cancer compared to normal controls, benign prostatic hyperplasia, prostatitis, and localized or recurrent disease. In an animal model, prostate tumor cells injected next to human bones implanted in the limb of mice demonstrated IL-6 expression [62]. In addition to IL-6, the IL-6R has been identified in human normal prostate and prostate carcinoma tissue [63, 64].

The secretion of IL-6 by prostate cancer cells in the bone microenvironment may impact bone remodeling [reviewed in 65, 66]. IL-6 promotes osteoclastogenesis [67-69] most likely through increasing osteoclastogenic precursors. IL-6-mediated osteoclastogenesis is directly related to the level of gp130 present on the precursor cells [70]. It appears that IL-6-mediated

osteoclastogenesis is independent of promoting RANKL expression [71]. However, IL-6 has been shown to potentiate PTHrP-induced osteoclastogenesis [72, 73]. Administration of anti-IL-6 antibody has been shown to diminish growth of subcutaneously injected prostate cancer cells in nude mice, thus demonstrating the potential utility of this compound in clinical prostate cancer [74]. These results strongly suggest that IL-6 may serve as a therapeutic target for the osteolytic component of prostate cancer skeletal metastases.

Direct Mediators of Bone Resorption

Cathepsins

Once activated, osteoclasts resorb bone through secretion of a combination of proteases to resorb the non-mineralized matrix and acid to dissolve the hydroxyapatitic mineral [75]. Proteases that are important mediators of osteoclastic activity include cathepsins and metalloproteinases. Cathepsins can cleave bone proteins such as Type I collagen, osteopontin, and osteonectin [76]. Various cathepsins exist such as cathepsin B, K, D and L. Each cathepsin produces a different pattern of collagen and non-collagen protein degradation [77]. Overexpression of cathepsin K in the mouse results in accelerated bone turnover [78]; whereas knockout of cathepsin K results in retarded bone matrix degradation and osteopetrosis [79]. Prostate cancer cells themselves make cathepsin K [80]. In the case of breast cancer, there are conflicting reports, some say that breast cancer cells express cathepsin K [81]; whereas other reports say they do not [82]although other cathepsins, such as cathepsin D are present [83]. The presence of cathepsin D in metastatic breast cancer cells [84] or in the serum of men with prostate cancer [85] indicates an aggressive tumor. Several novel classes of cathepsin inhibitors have been designed and may provide novel therapeutic agents to target bone resorption [86-88]. For example, CLIK-148, a cathepsin L inhibitor, has been shown in animal models to prevent local tumor-induced bone invasion and also inhibit growth of tumor in bone at sites distant from the tumor inoculation [86].

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs), a family of enzymes whose primary function is to degrade the extracellular matrix, play a role in bone remodeling. This activity occurs in the absence of osteoclasts [89] suggesting that MMPs have a direct resorptive effect. Several have the ability to degrade the non-mineralized matrix of bone including MMP-1, MMP-9 and MMP-13, which are collagenases. Other MMPs such as stromelysin (MMP-3) activate MMP-1. Through their proteolytic activity MMPs contribute to metastatic invasion, including destruction of bone [90].

Prostate carcinomas and their cell lines express a large number of MMPs [91-98]. Levels of MMP-9 secretion in primary prostate cancer cultures increased with Gleason histological grade [93]. Active MMP-9 species were detected in 15 cultures (31%) of primary prostate cancer tissues. The presence of the mineralized matrix has been shown to induce MMP-9 expression from prostate carcinoma cells [99].

The initial functional data that suggested prostate carcinoma bone metastasis modulate bone remodeling through MMPs was provided by in vitro studies. Specifically, blocking MMP activity with 1,10-phenanthroline, a MMP inhibitor, diminished bone matrix degradation induced by PC-3 cells in vitro [100, 101]. Matrilysin (MMP-7) has been shown to be upregulated in DU-145 prostate cancer cells and can enhance their invasive ability. Monoclonal antibody targeting the cytokine interleukin-6 (IL-6) has been shown to increase promatrilyisn expression in DU-145 cultures [102]. This suggests that IL-6, which is increased in prostate cancer [reviewed in 103], enhances prostate cancer invasion through production of MMP-7.

The importance of MMPs in bone metastasis has been further confirmed in vivo. An MMP inhibitor, batimistat, has been shown to inhibit development bone resorption in vitro and in vivo in murine models of breast [104] and prostate carcinoma [105]. The mechanism through which prostate carcinoma-produced MMPs induce bone resorption is not clear; however, it appears to involve induction of osteoclastogenesis as inhibition of MMPs reduced the number of osteoclasts associated with prostate tumor growth in human bone implants in mice [105]. Additionally, the bisphosphonate alendronate blocked MMP production from PC-3 cells [106]. This was associated with diminished establishment of bone metastasis in mice injected with PC-3 tumors [89].

Acid Secretion

In addition to the proteases, acid is secreted from osteoclasts to resorb the mineralized matrix. Acid is believed to be secreted through vacuolar H(+)-ATPase-dependent pumps present on the osteoclasts ruffled membranes [107]. Several hormones regulate acid secretion, including parathyroid hormone, which increases acid secretion and calcitonin, which decreases acid secretion. Carbonic anhydrase II appears to be an important mediator of acid production because acetazolamide, a carbonic anhydrase inhibitor-based diuretic, can block bone resorption [108]. Another diuretic, indapamide, increased osteoblast proliferation and decreased bone resorption, at least in part, by decreasing osteoclast differentiation via a direct effect on hematopoietic precursors in vitro [109]. These findings suggest that targeting osteoclast-derived activity, as in addition to targeting osteoclast production or survival, may provide therapeutic avenues to diminish tumor-induced bone resorption.

Conclusions

Prostate cancer skeletal metastases promote osteolysis through several mechanisms that include both activation of osteoclast-mediated bone resorption and direct resorption on non-mineralized bone matrix (Fig. 3). Delineating the mechanisms that promote prostate cancer skeletal metastasis and the interactions between metastatic prostate cancer cells and bones should lead to development of therapies that will diminish or prevent these events. Our current understanding of the biology of prostate cancer skeletal metastases has led to identification of several putative targets and therapies aimed at these targets, some of which are currently in clinical trials at the time of this writing. Continued research into the biology

of prostate cancer skeletal metastases should enable development of improved therapeutic regimens to diminish this painful aspect of prostate cancer.

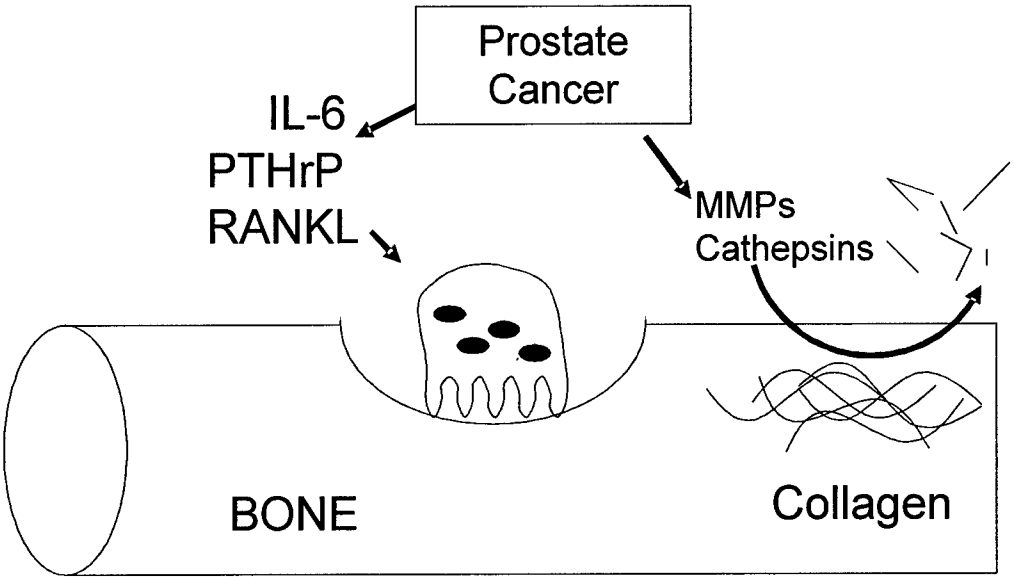

Figure 3. Mechanisms of prostate cancer metastases-mediated osteolysis.

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References

[1] Uy HL, Mundy GR, Boyce BF, Story BM, Dunstan CR, Yin JJ, Roodman GD, Guise TA. Tumor necrosis factor enhances parathyroid hormone-related protein- induced hypercalcemia and bone resorption without inhibiting bone formation in vivo. *Cancer Res* 1997;57(15):3194-3199.

[2] Lum L, Wong BR, Josien R, Becherer JD, Erdjument-Bromage H, Schlondorff J, Tempst P, Choi Y, Blobel CP. Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)- converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *J Biol Chem* 1999;274(19):13613-13618.

[3] Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J,

Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998;93(2):165-176.

[4] Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; 397(6717):315-323.

[5] Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998;95(7):3597-3602.

[6] Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. *J Exp Med* 1998;188(5):997-1001.

[7] Shalhoub V, Elliott G, Chiu L, Manoukian R, Kelley M, Hawkins N, Davy E, Shimamoto G, Beck J, Kaufman SA, Van G, Scully S, Qi M, Grisanti M, Dunstan C, Boyle WJ, Lacey DL. Characterization of osteoclast precursors in human blood. *Br J Haematol* 2000;111(2):501-512.

[8] Matsuo K, Owens JM, Tonko M, Elliott C, Chambers TJ, Wagner EF. Fosl1 is a transcriptional target of c-Fos during osteoclast differentiation. *Nat Genet* 2000;24(2):184-187.

[9] Hofbauer LC, Heufelder AE. The role of osteoprotegerin and receptor activator of nuclear factor kappaB ligand in the pathogenesis and treatment of rheumatoid arthritis. *Arthritis Rheum* 2001;44(2):253-259.

[10] Menaa C, Kurihara N, Roodman GD. CFU-GM-derived cells form osteoclasts at a very high efficiency. *Biochem Biophys Res Commun* 2000;267(3):943-946.

[11] Hofbauer LC, Neubauer A, Heufelder AE. Receptor activator of nuclear factor-kappaB ligand and osteoprotegerin: potential implications for the pathogenesis and treatment of malignant bone diseases. *Cancer* 2001;92(3):460-470.

[12] Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravallese EM. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. *Am J Pathol* 2001;159(5):1689-1699.

[13] Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. *Proc Natl Acad Sci U S A* 2000;97(4):1566-1571.

[14] Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Boyle WJ, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997;89(2):309-319.

[15] Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, Higashio K. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochem Biophys Res Commun* 1997;234(1):137-142.

[16] Tan KB, Harrop J, Reddy M, Young P, Terrett J, Emery J, Moore G, Truneh A. Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and non-hematopoietic cells. *Gene* 1997;204(1-2):35-46.

[17] Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology* 1998;139(3):1329-1337.

[18] Yun TJ, Chaudhary PM, Shu GL, Frazer JK, Ewings MK, Schwartz SM, Pascual V, Hood LE, Clark EA. OPG/FDCR-1, a TNF receptor family member, is expressed in lymphoid cells and is up-regulated by ligating CD40. *J Immunol* 1998;161(11):6113-6121.

[19] Hofbauer LC, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2, and cytokines. *Biochem Biophys Res Commun* 1998;250(3):776-781.

[20] Hofbauer LC, Heufelder AE. Osteoprotegerin and its cognate ligand: a new paradigm of osteoclastogenesis. *Eur J Endocrinol* 1998;139(2):152-154.

[21] Vidal NO, Brandstrom H, Jonsson KB, Ohlsson C. Osteoprotegerin mRNA is expressed in primary human osteoblast-like cells: down-regulation by glucocorticoids. *J Endocrinol* 1998;159(1):191-195.

[22] Kartsogiannis V, Zhou H, Horwood NJ, Thomas RJ, Hards DK, Quinn JM, Niforas P, Ng KW, Martin TJ, Gillespie MT. Localization of RANKL (receptor activator of NF kappa B ligand) mRNA and protein in skeletal and extraskeletal tissues. *Bone* 1999;25(5):525-534.

[23] Nagai M, Sato N. Reciprocal gene expression of osteoclastogenesis inhibitory factor and osteoclast differentiation factor regulates osteoclast formation. *Biochem Biophys Res Commun* 1999;257(3):719-723.

[24] Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR. The effect of a single dose of osteoprotegerin in postmenopausal women. *J Bone Miner Res* 2001;16(2):348-360.

[25] Thomas GP, Baker SU, Eisman JA, Gardiner EM. Changing RANKL/OPG mRNA expression in differentiating murine primary osteoblasts. *J Endocrinol* 2001;170(2):451-460.

[26] Fazzalari NL, Kuliwaba JS, Atkins GJ, Forwood MR, Findlay DM. The ratio of messenger RNA levels of receptor activator of nuclear factor kappaB ligand to osteoprotegerin correlates with bone remodeling indices in normal human cancellous bone but not in osteoarthritis. *J Bone Miner Res* 2001;16(6):1015-1027.

[27] Lin DL, Tarnowski CP, Zhang J, Dai J, Rohn E, Patel AH, Morris MD, Keller ET. Bone metastatic LNCaP-derivative C4-2B prostate cancer cell line mineralizes in vitro. *Prostate* 2001;47(3):212-221.

[28] Zhang J, Dai J, Qi Y, Lin DL, Smith P, Strayhorn C, Mizokami A, Fu Z, Westman J, Keller ET. Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. *J Clin Invest* 2001;107(10):1235-1244.

[29] Brown JM, Corey E, Lee ZD, True LD, Yun TJ, Tondravi M, Vessella RL. Osteoprotegerin and rank ligand expression in prostate cancer. *Urology* 2001;57(4):611-616.

[30] Brown JM, Vessella RL, Kostenuik PJ, Dunstan CR, Lange PH, Corey E. Serum osteoprotegerin levels are increased in patients with advanced prostate cancer. *Clin Cancer Res* 2001;7(10):2977-2983.

[31] Strewler GJ. The physiology of parathyroid hormone-related protein. *N Engl J Med* 2000;342(3):177-185.

[32] Iwamura M, Abrahamsson PA, Foss KA, Wu G, Cockett AT, Deftos LJ. Parathyroid hormone-related protein: a potential autocrine growth regulator in human prostate cancer cell lines. *Urology* 1994;43(5):675-679.

[33] Deftos LJ. Prostate carcinoma: production of bioactive factors. *Cancer* 2000;88(12 Suppl):3002-3008.

[34] Iwamura M, di Sant'Agnese PA, Wu G, Benning CM, Cockett AT, Deftos LJ, Abrahamsson PA. Immunohistochemical localization of parathyroid hormone-related protein in human prostate cancer. *Cancer Res* 1993;53(8):1724-1726.

[35] Asadi F, Farraj M, Sharifi R, Malakouti S, Antar S, Kukreja S. Enhanced expression of parathyroid hormone-related protein in prostate cancer as compared with benign prostatic hyperplasia. *Hum Pathol* 1996;27(12):1319-1323.

[36] Iwamura M, Gershagen S, Lapets O, Moynes R, Abrahamsson PA, Cockett AT, Deftos LJ, di Sant'Agnese PA. Immunohistochemical localization of parathyroid hormone-related protein in prostatic intraepithelial neoplasia. *Hum Pathol* 1995;26(7):797-801.

[37] Dougherty KM, Blomme EA, Koh AJ, Henderson JE, Pienta KJ, Rosol TJ, McCauley LK. Parathyroid hormone-related protein as a growth regulator of prostate carcinoma. *Cancer Res* 1999;59(23):6015-6022.

[38] Iddon J, Bundred NJ, Hoyland J, Downey SE, Baird P, Salter D, McMahon R, Freemont AJ. Expression of parathyroid hormone-related protein and its receptor in bone metastases from prostate cancer. *J Pathol* 2000;191(2):170-174.

[39] Kremer R, Goltzman D, Amizuka N, Webber MM, Rhim JS. ras Activation of human prostate epithelial cells induces overexpression of parathyroid hormone-related peptide. *Clin Cancer Res* 1997;3(6):855-859.

[40] Burton PB, Moniz C, Knight DE. Parathyroid hormone related peptide can function as an autocrine growth factor in human renal cell carcinoma. *Biochem Biophys Res Commun* 1990;167(3):1134-1138.

[41] Bouizar Z, Spyratos F, De vernejoul MC. The parathyroid hormone-related protein (PTHrP) gene: use of downstream TATA promotor and PTHrP 1-139 coding pathways in primary breast cancers vary with the occurrence of bone metastasis. *J Bone Miner Res* 1999;14(3):406-414.

[42] Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massague J, Mundy GR, Guise TA. TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *J Clin Invest* 1999;103(2):197-206.

[43] Peehl DM, Edgar MG, Cramer SD, Deftos LJ. Parathyroid hormone-related protein is not an autocrine growth factor for normal prostatic epithelial cells. *Prostate* 1997;31(1):47-52.

[44] Henderson JE, Amizuka N, Warshawsky H, Biasotto D, Lanske BM, Goltzman D, Karaplis AC. Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. *Mol Cell Biol* 1995;15(8):4064-4075.

[45] Aarts MM, Rix A, Guo J, Bringhurst R, Henderson JE. The nucleolar targeting signal (NTS) of parathyroid hormone related protein mediates endocytosis and nucleolar translocation. *J Bone Miner Res* 1999;14(9):1493-1503.

[46] Ye Y, Wang C, Du P, Falzon M, Seitz PK, Cooper CW. Overexpression of parathyroid hormone-related protein enhances apoptosis in the rat intestinal cell line, IEC-6. *Endocrinology* 2001;142(5):1906-1914.

[47] Massfelder T, Dann P, Wu TL, Vasavada R, Helwig JJ, Stewart AF. Opposing mitogenic and anti-mitogenic actions of parathyroid hormone-related protein in vascular smooth muscle cells: a critical role for nuclear targeting. *Proc Natl Acad Sci U S A* 1997;94(25):13630-13635.

[48] Blomme EA, Dougherty KM, Pienta KJ, Capen CC, Rosol TJ, McCauley LK. Skeletal metastasis of prostate adenocarcinoma in rats: morphometric analysis and role of parathyroid hormone-related protein. *Prostate* 1999;39(3):187-197.

[49] Cramer SD, Chen Z, Peehl DM. Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. *J Urol* 1996;156(2 Pt 1):526-531.

[50] Rabbani SA, Gladu J, Harakidas P, Jamison B, Goltzman D. Over-production of parathyroid hormone-related peptide results in increased osteolytic skeletal metastasis by prostate cancer cells in vivo. *Int J Cancer* 1999;80(2):257-264.

[51] Sehgal P, Wang L, Rayanade R, Pan H, Margulies L. Interleukin-6 type Cytokines. In: Mackiewicz A, Koji A, Sehgal P, editors. *Ann N Y Acad Sci.* Volume 762. New York: New York Academy of Sciences; 1995. p 1-14.

[52] Hirano T. The biology of interleukin-6. *Chem Immunol* 1992;51:153-180.

[53] Taga T, Hibi M, Murakami M, Saito M, Yawata H, Hirano T, Kishimoto T. Interleukin-6 receptor and signals. In: Kishimoto T, editor. Interleukins: *Molecular Biology and Immunology Chem Immunol.* Volume 51: Basel, Karger; 1992. p 181-204.

[54] Mullberg J, Oberthur W, Lottspeich F, Mehl E, Dittrich E, Graeve L, Heinrich PC, Rose-John S. The soluble human IL-6 receptor. Mutational characterization of the proteolytic cleavage site. *J Immunol* 1994;152(10):4958-4968.

[55] Rose-John S, Heinrich PC. Soluble receptors for cytokines and growth factors: generation and biological function. *Biochem J* 1994;300(Pt 2):281-290.

[56] Lust JA, Donovan KA, Kline MP, Greipp PR, Kyle RA, Maihle NJ. Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. *Cytokine* 1992;4(2):96-100.

[57] Mackiewicz A, Schooltink H, Heinrich PC, Rose-John S. Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. *J Immunol* 1992;149(6):2021-2027.

[58] Adler HL, McCurdy MA, Kattan MW, Timme TL, Scardino PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor-beta1 in patients with metastatic prostatic carcinoma. *J Urol* 1999;161(1):182-187.

[59] Hoosien N, Abdul M, McCabe R, Gero E, Deftos L, Banks M, Hodges S, Finn L, Logothetis C. Clinical significance of elevation in neuroendocrine factors and interleukin-6 in metastatic prostate cancer. *Urol Oncol* 1995;1:246-251.

[60] Twillie DA, Eisenberger MA, Carducci MA, Hseih WS, Kim WY, Simons JW. Interleukin-6: a candidate mediator of human prostate cancer morbidity. *Urology* 1995;45(3):542-549.

[61] Drachenberg DE, Elgamal AA, Rowbotham R, Peterson M, Murphy GP. Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. *Prostate* 1999;41(2):127-133.

[62] Tsingotjidou AS, Zotalis G, Jackson KR, Sawyers C, Puzas JE, Hicks DG, Reiter R, Lieberman JR. Development of an animal model for prostate cancer cell metastasis to adult human bone. *Anticancer Res* 2001;21(2A):971-978.

[63] Siegsmund MJ, Yamazaki H, Pastan I. Interleukin 6 receptor mRNA in prostate carcinomas and benign prostate hyperplasia. *J Urol* 1994;151(5):1396-1399.

[64] Hobisch A, Rogatsch H, Hittmair A, Fuchs D, Bartsch G, Jr., Klocker H, Bartsch G, Culig Z. Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *J Pathol* 2000;191(3):239-244.

[65] Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty [In Process Citation]. *Annu Rev Med* 2000;51:245-270.

[66] Manolagas SC. The role of IL-6 type cytokines and their receptors in bone. *Ann N Y Acad Sci* 1998;840:194-204.

[67] Jilka RL, Passeri G, Girasole G, Cooper S, Abrams J, Broxmeyer H, Manolagas SC. Estrogen loss upregulates hematopoiesis in the mouse: a mediating role of IL-6. *Exp Hematol* 1995;23(6):500-506.

[68] Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Costantini F. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO Journal* 1994;13(5):1189-1196.

[69] de Grooth R, Kawilarang-de Haas EW, van de Sande-Rijkers CM, Nijweide PJ. The role of osteoblast density and endogenous interleukin-6 production in osteoclast formation from the hemopoietic stem cell line FDCP-MIX C2GM in coculture with primary osteoblasts. *Calcif Tissue Int* 1998;63(1):57-62.

[70] O'Brien CA, Lin SC, Bellido T, Manolagas SC. Expression levels of gp130 in bone marrow stromal cells determine the magnitude of osteoclastogenic signals generated by IL-6-type cytokines. *J Cell Biochem* 2000;79(4):532-541.

[71] Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin- 6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. *Bone* 1999;25(3):255-259.

[72] Greenfield EM, Shaw SM, Gornik SA, Banks MA. Adenyl cyclase and interleukin 6 are downstream effectors of parathyroid hormone resulting in stimulation of bone resorption. *J Clin Invest* 1995;96(3):1238-1244.

[73] de la Mata J, Uy HL, Guise TA, Story B, Boyce BF, Mundy GR, Roodman GD. Interleukin-6 enhances hypercalcemia and bone resorption mediated by parathyroid hormone-related protein in vivo. *J Clin Invest* 1995;95(6):2846-2852.

[74] Smith PC, Keller ET. Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. *Prostate* 2001;48:47-53.

[75] Blair HC. How the osteoclast degrades bone. *Bioessays* 1998;20(10):837-846.

[76] Katunuma N. Mechanism and regulation of bone resorption by osteoclasts. *Curr Top Cell Regul* 1997;35:179-192.

[77] Page AE, Hayman AR, Andersson LM, Chambers TJ, Warburton MJ. Degradation of bone matrix proteins by osteoclast cathepsins. *Int J Biochem* 1993;25(4):545-550.

[78] Kiviranta R, Morko J, Uusitalo H, Aro HT, Vuorio E, Rantakokko J. Accelerated turnover of metaphyseal trabecular bone in mice overexpressing cathepsin K. *J Bone Miner Res* 2001;16(8):1444-1452.

[79] Gowen M, Lazner F, Dodds R, Kapadia R, Feild J, Tavaria M, Bertoncello I, Drake F, Zavarselk S, Tellis I, Hertzog P, Debouck C, Kola I. Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. *J Bone Miner Res* 1999;14(10):1654-1663.

[80] Brubaker KD, Vessella RL, True LD, Thomas R, Corey E. Cathepsin K mRNA and protein expression in prostate cancer progression. *J Bone Miner Res* 2003;18(2):222-230.

[81] Littlewood-Evans AJ, Bilbe G, Bowler WB, Farley D, Wlodarski B, Kokubo T, Inaoka T, Sloane J, Evans DB, Gallagher JA. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Res* 1997;57(23):5386-5390.

[82] Ishibashi O, Mori Y, Kurokawa T, Kumegawa M. Breast cancer cells express cathepsins B and L but not cathepsins K or H. *Cancer Biochem Biophys* 1999;17(1-2):69-78.

[83] James BA, Cranor ML, Rosen PP. Carcinoma of the breast arising in microglandular adenosis. *Am J Clin Pathol* 1993;100(5):507-513.

[84] Solomayer EF, Diel IJ, Meyberg GC, Gollan C, Bode S, Wallwiener D, Bastert G. Prognostic relevance of cathepsin D detection in micrometastatic cells in the bone marrow of patients with primary breast cancer. *Breast Cancer Res Treat* 1998;49(2):145-154.

[85] Hara I, Miyake H, Yamanaka K, Hara S, Kamidono S. Serum cathepsin D and its density in men with prostate cancer as new predictors of disease progression. *Oncol Rep* 2002;9(6):1379-1383.

[86] Katunuma N, Tsuge H, Nukatsuka M, Asao T, Fukushima M. Structure-based design of specific cathepsin inhibitors and their application to protection of bone metastases of cancer cells. *Arch Biochem Biophys* 2002;397(2):305-311.

[87] Yamashita DS, Dodds RA. Cathepsin K and the design of inhibitors of cathepsin K. *Curr Pharm Des* 2000;6(1):1-24.

[88] Stroup GB, Lark MW, Veber DF, Bhattacharyya A, Blake S, Dare LC, Erhard KF, Hoffman SJ, James IE, Marquis RW, Ru Y, Vasko-Moser JA, Smith BR, Tomaszek T, Gowen M. Potent and selective inhibition of human cathepsin K leads to inhibition of bone resorption in vivo in a nonhuman primate. *J Bone Miner Res* 2001;16(10):1739-1746.

[89] Stearns ME, Wang M. Effects of alendronate and taxol on PC-3 ML cell bone metastases in SCID mice. *Invasion Metastasis* 1996;16(3):116-131.

[90] John A, Tuszynski G. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. *Pathol Oncol Res* 2001;7(1):14-23.

[91] Boag AH, Young ID. Immunohistochemical analysis of type IV collagenase expression in prostatic hyperplasia and adenocarcinoma. *Mod Pathol* 1993;6(1):65-68.

[92] Bodey B, Bodey B, Jr., Siegel SE, Kaiser HE. Immunocytochemical detection of matrix metalloproteinase expression in prostate cancer. *In Vivo* 2001;15(1):65-70.

[93] Festuccia C, Bologna M, Vicentini C, Tacconelli A, Miano R, Violini S, Mackay AR. Increased matrix metalloproteinase-9 secretion in short-term tissue cultures of prostatic tumor cells. *Int J Cancer* 1996;69(5):386-393.

[94] Hamdy FC, Fadlon EJ, Cottam D, Lawry J, Thurrell W, Silcocks PB, Anderson JB, Williams JL, Rees RC. Matrix metalloproteinase 9 expression in primary human prostatic adenocarcinoma and benign prostatic hyperplasia. *Br J Cancer* 1994;69(1):177-182.

[95] Hashimoto K, Kihira Y, Matuo Y, Usui T. Expression of matrix metalloproteinase-7 and tissue inhibitor of metalloproteinase-1 in human prostate. *J Urol* 1998;160(5):1872-1876.

[96] Montironi R, Fabris G, Lucarini G, Biagini G. Location of 72-kd metalloproteinase (type IV collagenase) in untreated prostatic adenocarcinoma. *Pathol Res Pract* 1995;191(11):1140-1146.

[97] Montironi R, Lucarini G, Castaldini C, Galluzzi CM, Biagini G, Fabris G. Immunohistochemical evaluation of type IV collagenase (72-kd metalloproteinase) in prostatic intraepithelial neoplasia. *Anticancer Res* 1996;16(4A):2057-2062.

[98] Pajouh MS, Nagle RB, Breathnach R, Finch JS, Brawer MK, Bowden GT. Expression of metalloproteinase genes in human prostate cancer. *J Cancer Res Clin Oncol* 1991;117(2):144-150.

[99] Festuccia C, Giunciuglio D, Guerra F, Villanova I, Angelucci A, Manduca P, Teti A, Albini A, Bologna M. Osteoblasts modulate secretion of urokinase-type plasminogen activator (uPA) and matrix metalloproteinase-9 (MMP-9) in human prostate cancer cells promoting migration and matrigel invasion. *Oncol Res* 1999;11(1):17-31.

[100] Duivenvoorden WC, Hirte HW, Singh G. Use of tetracycline as an inhibitor of matrix metalloproteinase activity secreted by human bone-metastasizing cancer cells. *Invasion Metastasis* 1997;17(6):312-322.

[101] Sanchez-Sweatman OH, Orr FW, Singh G. Human metastatic prostate PC3 cell lines degrade bone using matrix metalloproteinases. *Invasion Metastasis* 1998;18(5-6):297-305.

[102] Stratton MS, Sirvent H, Udayakumar TS, Nagle RB, Bowden GT. Expression of the matrix metalloproteinase promatrilysin in coculture of prostate carcinoma cell lines. *Prostate* 2001;48(3):206-209.

[103] Smith PC, Hobish A, Lin D, Culig Z, Keller ET. Interleukin-6 and prostate cancer progression. *Cytokine Growth Factor Rev* 2001;12:33-40.

[104] Lee J, Weber M, Mejia S, Bone E, Watson P, Orr W. A matrix metalloproteinase inhibitor, batimastat, retards the development of osteolytic bone metastases by MDA-MB-231 human breast cancer cells in Balb C nu/nu mice. *Eur J Cancer* 2001;37(1):106-113.

[105] Nemeth JA, Yousif R, Herzog M, Che M, Upadhyay J, Shekarriz B, Bhagat S, Mullins C, Fridman R, Cher ML. Matrix metalloproteinase activity, bone matrix turnover, and tumor cell proliferation in prostate cancer bone metastasis. *J Natl Cancer Inst* 2002;94(1):17-25.

[106] Stearns ME, Wang M. Alendronate blocks metalloproteinase secretion and bone collagen I release by PC-3 ML cells in SCID mice. *Clin Exp Metastasis* 1998;16:693-702.

[107] Lee BS, Holliday LS, Ojikutu B, Krits I, Gluck SL. Osteoclasts express the B2 isoform of vacuolar H(+)-ATPase intracellularly and on their plasma membranes. *Am J Physiol* 1996;270(1 Pt 1):C382-388.

[108] Lehenkari P, Hentunen TA, Laitala-Leinonen T, Tuukkanen J, Vaananen HK. Carbonic anhydrase II plays a major role in osteoclast differentiation and bone resorption by effecting the steady state intracellular pH and Ca2+. *Exp Cell Res* 1998;242(1):128-137.

[109] Lalande A, Roux S, Denne MA, Stanley ER, Schiavi P, Guez D, De Vernejoul MC. Indapamide, a thiazide-like diuretic, decreases bone resorption in vitro. *J Bone Miner Res* 2001;16(2):361-370.

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Prostate Cancer Bone Metastases Promote Both Osteolytic and Osteoblastic Activity

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Abstract Advanced prostate cancer is frequently accompanied by the development of metastasis to bone. In the past, prostate cancer bone metastases were characterized as being osteoblastic (i.e., increasing bone density) based on radiographs. However, emerging evidence suggests that development of prostate cancer bone metastases requires osteoclastic activity in addition to osteoblastic activity. The complexities of how prostate tumor cells influence bone remodeling are just beginning to be elucidated. Prostate cancer cells produce a variety of pro-osteoblastic factors that promote bone mineralization. For example, both bone morphogenetic proteins and endothelin-1 have well recognized pro-osteoblastic activities and are produced by prostate cancer cells. In addition to factors that enhance bone mineralization prostate cancer cells produced factors that promote osteoclast activity. Perhaps the most critical pro-osteoclastogenic factor produced by prostate cancer cells is receptor activator of NFκB ligand (RANKL), which has been shown to be required for the development of osteoclasts. Blocking RANKL results in inhibiting prostate cancer-induced osteoclastogenesis and inhibits development and progression of prostate tumor growth in bone. These findings suggest that targeting osteoclast activity may be of therapeutic benefit. However, it remains to be defined how prostate cancer cells synchronize the combination of osteoclastic and osteoblastic activity. We propose that as the bone microenvironment is changed by the developing cancer, this in turn influences the prostate cancer cells' balance between pro-osteoclastic and pro-osteoblastic activity. Accordingly, the determination of how the prostate cancer cells and bone microenvironment crosstalk are important to elucidate how prostate cancer cells modulate bone remodeling. J. Cell. Biochem. 91: 718–729, 2004.

Key words: prostate cancer; bone metastases; metastasis; bone remodeling; OPG; BMP; ET-1; RANKL

Bone is the most frequent site of prostate carcinoma metastasis with skeletal metastases identified at autopsy in up to 90% of patients dying from prostate carcinoma [Abrams et al., 1950; Rana et al., 1993; Bubendorf et al., 2000]. Skeletal metastasis results in significant complications including bone pain, impaired mobility, pathological fracture, spinal cord compression, and symptomatic hypercalcemia [Galasko, 1986; Coleman, 1997; Moul and Lipo, 1999]. Despite advances in the diagnosis and management of prostate carcinoma, advanced disease with skeletal metastasis remains incurable. Current therapeutic modalities are mostly palliative, and include hormonal therapy, pharmacological management of bone pain, radiotherapy for pain, and spinal cord compression [Szostak and Kyprianou, 2000], various chemotherapy regimens, and the use of bisphosphonates to inhibit osteoclast activity [Papapoulos et al., 2000]. In spite of the severe complications of prostate carcinoma skeletal metastasis, there has not been much advance in the therapeutic arena to prevent or diminish these lesions. It is critical that a solid understanding of the pathophysiology of prostate carcinoma skeletal metastatic process is

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developed to provide the basis for creating strategies to prevent or diminish their occurrence and associated complications.

There are many challenges that encompass determining the mechanisms that contribute to the selective development of CaP in bone [Lange and Vessella, 1998; Rosol, 2000]. These include mechanisms of homing to bone and tumor cell attachment at the bone endothelial site. However, once in the bone, CaP tumors have pathobiology that appears to be somewhat unique to cancer skeletal metastases. Specifically, CaP skeletal metastases are most often radiographically characterized as osteoblastic (i.e., increased mineral density at the site of the lesion) as opposed to osteolytic. Other tumors, such as breast cancer, can form osteoblastic lesions; however, these occur less frequently [Munk et al., 1997; Yamashita et al., 2000]. In spite of the radiographic osteoblastic appearance it is clear from histological evidence that CaP metastases form a heterogeneous mixture of osteolytic and osteoblastic lesions although osteoblastic lesions are predominant [Urwin et al., 1985; Percival et al., 1987; Berruti et al., 1996; Vinholes et al., 1996; Roudier et al., 2000]. Recent evidence shows that osteoblastic metastases form on trabecular bone at sites of previous osteoclastic resorption, and that such resorption may be required for subsequent osteoblastic bone formation [Carlin and Andriole, 2000; Zhang et al., 2001]. These findings suggest that CaP induces bone production through an overall increase in bone remodeling, which in the nonpathologic state is a balance between osteoclast resorption of bone, followed by osteoblast-mediated replacement of resorbed bone (reviwed in Boyce et al., 1999a; Karsenty, 2000; Parfitt, 2000). The mechanisms through which CaP cells promote bone mineralization or bone resorption remain poorly understood. Dissecting these mechanisms should help identify molecular targets for therapeutic approaches to prevent the damaging effects of CaP on the skeleton and their associated complications.

THE PRO-OSTEOBLASTIC NATURE OF PROSTATE CANCER

Histomorphometric evidence indicates that sites of prostate carcinoma bone metastases often have microscopic evidence of increased bone production including increased osteoid surface, osteoid volume, and mineralization rates [Charhon et al., 1983; Clarke et al., 1993]. The histological findings are consistent with clinical evidence that demonstrates increased systemic markers of both bone production in prostate carcinoma patients [Maeda et al., 1997; Demers et al., 2000]. However, evidence that osteoclast activity occurs is also found, which suggests that prostate carcinoma induces bone production through an overall increase in bone remodeling. In the case of prostate carcinoma, it appears the induction of osteoblast-mediated mineralization eventually outweighs the increase in osteoclast resorption resulting in an overall formation of osteoblastic lesions. Although it would seem that the increased bone production would not decrease the bones mechanical properties (i.e., its strength) it actually weakens the bone for the following reasons; mature, healthy bone is formed of lamellar bone, which consists of collagen bundles that are organized in a tightly packed linear fashion resulting in optimum bone strength. In contrast, prostate carcinoma induces production of woven bone, which is composed of loosely packed, randomly oriented collagen bundles that produce bone with suboptimal strength [Blomme et al., 1999; Rosol, 2000]. The combination of inferior bone production and underlying osteolysis leads to a predisposition to fracture.

The mechanisms through which prostate carcinoma cells promote bone mineralization remain poorly understood. However, prostate carcinoma cells produce a variety of factors that have direct or indirect osteogenic properties (Table I) (reviewed in Goltzman et al., 1992; Yoneda, 1998; Boyce et al., 1999b; Deftos, 2000). Some of these factors, such as bone morphogenetic proteins (BMP) [Harris et al., 1994; Autzen et al., 1998; Hullinger et al., 2000] and enodothlin-1 (ET-1) [Nelson et al., 1995] may directly stimulate differentiation of osteoblast precursors to mature mineral-producing osteoblasts [Kimura et al., 1992] or induce osteoblast protein production [Hullinger et al., 2000]. Other factors such as parathyroid hormone-related protein (PTHrP) may work through inhibition of osteoblast apoptosis) [Karaplis and Vautour, 1997; Cornish et al., 1999]. Additionally, there are proteins that may work indirectly to enhance bone production, such as the serine proteases, prostate specific antigen (PSA), and urinary plasminogen activator (uPA), which can activate latent forms of osteogenic proteins, such as transforming growth

TABLE I. Osteogenic Factors Produced by Cancer Cells

Factor	Reference
Bone morphogenetic proteins (BMP)	[Bentley et al., 1992; Hullinger et al., 2000]
Endothelin-1 (ET-1)	[Nelson et al., 1995; Nelson and Carducci, 2000]
Insulin-like growth factors (IGF)	[Perkel et al., 1990; Pirtskhalaishvili and Nelson, 2000]
Interleukin-1 and -6	[Taguchi et al., 1998; Le Brun et al., 1999]
Osteoprotegerin (OPG)	[Guise, 2000; Honore et al., 2000]
Parathyroid hormone-related peptide (PTHrP)	[Karaplis and Vautour, 1997; Cornish et al., 1999]
Transforming growth factor-β(TFG-β)	[Killian et al., 1993]
Urinary plasminogen activator (urokinase)	[Goltzman et al., 2000]

factor-β (TFG-β) [Killian et al., 1993; Rabbani et al., 1997]. Finally, some molecules, such as osteoprotegerin (OPG) [Simonet et al., 1997; Guise, 2000; Honore et al., 2000; Lee et al., 2003] and ET-1 (in a dual role with its osteoblast-stimulating activity) [Chiao et al., 2000] can enhance osteosclerosis through inhibiting osteoclastogenesis. Other tumor types, such as osteosarcoma, are also known to produce a variety of osteoblastic factors [Wlosarski and Reddi, 1987; Raval et al., 1996; Laitinen et al., 1998]. With such a large number of factors, it is difficult to determine which the key factor is, and most likely several of these osteogenic factors work in concert to produce maximal bone production. We will highlight two of the factors, BMP and endothelin-1 (ET-1), for which there is currently the most evidence for a role in prostate cancer-induced osteosclerosis.

BMP are members of the TFG-β superfamily. More than 30 BMPs have been identified to date [Ducy and Karsenty, 2000]. While originally discovered because of their ability to induced new bone formation, BMPs are now recognized to perform many functions, particularly in the role of development, such as apoptosis, differentiation, proliferation, and morphogenesis (reviewed in Hogan, 1996; Reddi, 1997; Hall and Miyake, 2000). BMPs are synthesized as large precursor molecules that undergo proteolytic cleavage to release the mature protein, which form active hetero- or homodimers [Wozney, 1992; Suzuki et al., 1997]. BMPs bind to receptors (BMPR-IA and -IB) and a BMP type II receptor (BMPR-II), which induces Smad phosphorylation [Wrana, 2000] resulting in modulation of gene regulation. Target genes of BMPs include osteoblast proteins such as OPG [Wan et al., 2001] and the osteoblast-specific transcription factor Cbfa-1 [Tsuji et al., 1998; Gori et al., 1999]. Several proteins that antagonize BMP action have been identified. For example, noggin and gremlin inhibit BMP-2, -4, and -7 by binding to them [Zimmerman et al., 1996; Merino et al., 1999; Abe et al., 2000]. Furthermore, the BMPs themselves regulate their own inhibitors in an apparent negative feedback mechanism [Nifuji and Noda, 1999; Nifuji et al., 1999].

Many in vitro studies have demonstrated that BMPs induce osteogenic differentiation including the ability of BMP-7 (also called osteogenic protein-1; OP-1) to induce osteogenic differentiation of newborn rat calvarial cells and rat osteosarcoma cells [Asahina et al., 1993; Maliakal et al., 1994; Li et al., 1996]. The BMP's osteogenic properties appear to be specific to the differentiation stage of the target cells. Specifically, BMPs can induce uncommitted stem cells [Katagiri et al., 1990; Li et al., 1996; Yamaguchi et al., 1996] and myoblasts [Katagiri et al., 1997] to express osteoblast parameters such as alkaline phosphatase or osteocalcin expression [Ducy et al., 2000; Karsenty, 2000]; whereas, BMPs do not stimulate mature osteoblasts or fibroblasts [Knutsen et al., 1993; Yamaguchi et al., 1996; Kim et al., 1997; Groeneveld and Burger, 2000] to increase expression of these proteins. Examination of genetically modified mice provides further evidence of the importance of BMP in bone development. The *bmp7* homozygous null condition in mice is a postnatal lethal mutation and is associated with, in addition to renal and ocular abnormalities, retarded skeletal ossification [Jena et al., 1997]. In contrast, *bmp6* null mice are viable and fertile, and the skeletal elements of newborn and adult mutants are indistinguishable from wildtype [Solloway et al., 1998]. However, careful examination of skeletogenesis in late gestation embryos reveals a consistent delay in ossification strictly confined to the developing sternum. Finally, mice with mutations of the *bmp5* gene have skeletal abnormalities and inefficient fracture repair [Kingsley et al., 1992]. Thus, taken together, these data provide

evidence that BMPs are important regulators of the osteogenesis. Thus, dysregulation of their expression in the bone microenvironment would most likely impact bone remodeling.

A few studies have examined the expression of BMPs in normal and neoplastic prostate tissues. Using Northern analysis, Harris et al. [1994] examined for BMP-2, 3, 4, and 6 mRNA expression in human normal prostate and prostate carcinoma cell lines. They found that normal human prostate predominantly expressed BMP-4. The androgen-dependent non-metastatic LNCaP human prostate carcinoma cell line produced very low to undetectable levels of BMPs. Whereas, the aggressive androgen-independent PC-3 cell line expressed very high levels of BMP-3 and slightly lower levels of BMP-2, -4, and -6 compared to normal cells, but much higher than LNCaP cells. In support of these results, Weber et al. [1998], using PCR analysis, identified 16 (73%) of 22 prostate carcinoma samples were positive for BMP-7 mRNA compared to eight (57%) of 14 normal prostate tissue samples. In another PCR based analysis, Bentley et al. [1992], found that several BMPs were expressed in both benign and malignant prostate tissue and in the PC3 and DU145 prostate carcinoma cell lines. BMP-6 expression was detected in the prostate tissue of over 50% of patients with clinically defined metastatic prostate adenocarcinoma, but was not detected in non-metastatic or benign prostate samples. In another study focused on BMP-6 mRNA and protein expression, Barnes et al. [1995] observed that BMP-6 was produced by normal and neoplastic human prostate (radical prostatectomy specimens and human carcinoma cell lines DU145 and PC3). However, BMP-6 mRNA and protein expression was higher in prostate carcinoma as compared with adjacent normal prostate, with higher-grade tumors (Gleason score of 6 or more) having greater BMP-6 immunostaining than the lower-grade tumors (Gleason score of 4 or less). These results were consistent with a later study by Hamdy et al. [1997], who reported that BMP-6 mRNA expression was detected exclusively in malignant epithelial cells in 20 of 21 patients (95%) with metastases, in 2 of 11 patients (18%) with localized cancer, and undetectable in eight benign samples. Futhermore, BMP-7 mRNA levels were found to be higher in prostate cancer skeletal metastases than in bone itself [Masuda et al., 2003]. In addition to BMPs, there have been several reports on prostate carcinoma expression of BMPR, it appears that as prostate carcinoma progress, the cells down-regulate their own expression of BMPRs [Ide et al., 1997a; Kim et al., 2000], which may be a protective mechanism as it has been demonstrated that BMP-2 can inhibit prostate carcinoma cell proliferation [Ide et al., 1997b]. Taken together, these observations demonstrate that prostate carcinoma cells produce increasing levels of BMPs as they progress to a more aggressive phenotype and suggest that the upregulation of BMP expression in prostate carcinoma cells localized in the bone is a critical component of the mechanism of development of osteoblastic lesions at prostate carcinoma metastatic sites.

Endothelins

Osteoblastic metastases occur in most prostate cancers and frequently in other common malignancies, such as breast cancer [Guise and Mundy, 1998]. Many tumor-associated factors have been proposed as mediators of the disorganized new bone formation at sites of metastases, including insulin-like growth factors (IGF)-1 and -2, transforming growth factor (TGF) β, prostate-specific antigen (PSA), urokinase-type plasminogen activator (UPA), fibroblast growth factors (FGF)-1 and -2, BMPs, and endothelin-1 (ET-1) [Achbarou et al., 1994; Thalmann et al., 1994; Nelson et al., 1995, 1996, 1999; Gingrich et al., 1996].

Accumulating evidence implicate ET-1 in the pathogenesis of osteoblastic metastases. Yanagisawa et al. [1988] originally purified ET-1 from endothelial cells. ET-1 is a potent vasoconstrictor, belonging to a family of three 21-amino-acid peptides, with a variety of functions [La and Reid, 1995]. The endothelins mediate their effects through endothelin A (ETA) and endothelin B (ETB) receptors. ETA receptors bind ET-1 with ten times greater affinity than ET-3 while the B receptor binds all three endothelins with equal affinity.

ET-1 has multiple effects on bone cells. It stimulates mitogenesis in osteoblasts, which express both ETA and ETB receptors [Takuwa et al., 1990; Stern et al., 1995]. ET-1 decreases osteoclastic bone resorption and osteoclast motility [Alam et al., 1992]. Immunohistochemistry of bone detected ET-1 in osteocytes, osteoblasts, and osteoclasts [Sasaki and Hong, 1993a,b].

Nelson et al. [1995] suggested the link between osteoblastic metastases, prostate cancer, and ET-1. They demonstrated that plasma ET-1 concentrations were significantly higher in men with advanced, hormone-refractory prostate cancer with bone metastases compared to men with organ-confined prostate cancer or normal controls [Nelson et al., 1995]. However, ET-1 concentrations were not correlated to tumor burden in bone or to serum prostate-specific antigen (PSA) concentrations.

Prostatic epithelium produces ET-1, and high-affinity receptors are present throughout the prostate gland [Nelson et al., 1995, 1996, 1999]. A majority of prostate cancers at primary as well as at metastatic sites express ET-1. Exogenous ET-1 increases the proliferation of prostate cancer as well as augmenting the mitogenic effects of IGF-1, -2; platelet-derived growth factor (PDGF); epidermal growth factor (EGF) and FGF-2 on prostate cancer cells. These effects are mediated via ETA receptors [Nelson et al., 1996]. ETB receptor expression was decreased in cancerous compared to normal prostate and was low in the prostate cancer cell lines PC3, DU 145, and LNCaP.

Breast cancers also express ET-1 and are the next most common tumor to cause osteoblastic metastases. Human breast cancer cells MCF-7, T47-D, and MDA-MB-231 have been shown to express the endothelin-processing enzyme necessary to convert preproET-1 to ET-1 [Patel and Schrey, 1995; Schrey and Patel, 1995; Yorimitsu et al., 1995; Patel et al., 1997]. Thus, substantial data implicate ET-1 in the pathogenesis of osteoblastic metastases due to prostate and breast cancers. However, a direct demonstration of a causal role for ET-1 in bone metastasis has not previously been reported. Questions remain about whether ET-1 has effects on bone formation in vivo, about the specificity of its effects, and about whether the increase in ET-1 observed in patients with prostate cancer represents a causative factor.

The bulk of evidence for a pro-osteoblastic metastatic effect of ET-1 has been derived from breast cancer skeletal metastases. Recent evidence indicates that breast cancer lines (ZR-75-1, MCF-7, and T47D) all cause osteoblastic metastases in female nude mice and produce ET-1 [Yin et al., 2000]. Conditioned media from these cell lines, as well as exogenous ET-1, stimulated osteoblast proliferation and new bone formation in cultures of mouse calvariae. These effects were inhibited by nonselective and ETA, but not ETB, receptor antagonists. Mice inoculated with ZR-75-1 and treated with oral ABT-627, a selective ETA receptor antagonist (2 or 20 mg/kg/day), had significantly fewer bone metastases compared with untreated ZR-75-1-mice. Bone histomorphometry revealed that the untreated ZR-75-1-mice had greater total bone area as well as new bone area compared with ABT-627-treated ZR-75-1-mice at either dose. Tumor burden in bone was significantly less in ABT-627-treated mice. In contrast, there was no effect of ABT-627 on osteolytic bone metastases caused by ET-1-negative breast cancer, MDA-MB-231. ETA and ETB expression, determined by RT-PCR, revealed that ZR-75-1 expressed neither ETA nor ETB while MDA-MB-231 expressed both. There was no effect of ABT-627 on (1) in vitro growth of ZR-75-1 or MDA-MB-231 or (2) in vivo growth of ZR-75-1 or MDA-MB-231 mammary fat pad tumors. These data indicate that the effects of ABT-627 to inhibit osteoblastic metastases are not direct effects on these tumor cells, but rather directed against the osteoblastic response of tumor-produced ET-1. Collectively these data suggest that tumor-produced ET-1 likely has a major role in the establishment of osteoblastic bone metastases by stimulating osteoblast proliferation and new bone formation. In terms of prostate cancer, atrasentan, an antagonist of ET-1 receptor A, partially reversed primary murine osteoblast proliferation induced by prostate cancer cells [Fizazi et al., 2003], suggesting that ET-1 may play a role in vivo. Blockade of the ETA receptor may be useful for prevention and the treatment of osteoblastic bone metastases due to breast or prostate cancer.

In addition to production of pro-osteoblastic factors, prostate cancer cells themselves gain an osteoblast-like phenotype. The initial evidence for this possibility was shown in a study that demonstrated C4-2B prostate cancer cells mineralized in vitro [Lin et al., 2001]. Furthermore, increased nuclear expression of the bone-specific transcription factor Cbfa1 (also known as Runx2, CCD, AML3, CCD1, OSF2) was found in the C4-2B cells and blocking Cbfa1 activity decreased the ability of C4-2B cells to mineralize in vitro. Additionally, mRNA and protein of the osteoblast-active transcription factor Cbfa1 were detected in prostate cancer tissues and cell lines [Brubaker et al., 2003]. Finally, a

specific Cbfa1: OSE2 (an osteoblast-specific cis-acting element present in the osteocalcin promoter) complex could be formed with PC-3 nuclear extracts. These data suggest that prostate cancer cells may promote osteosclerosis directly, although direct evidence of this has not been provided to date.

In summary, a variety of factors may promote the osteoblastic nature of prostate cancer bone metastases. Most likely no individual factor is responsible for prostate cancer-induced osteosclerosis, but rather several factors work in concert to induce both osteoblastogenesis and osteoblast activity.

THE PRO-OSTEOLYTIC NATURE OF PROSTATE CANCER

In healthy adults, the regulated destruction (resorption or lysis) of normal lamellar bone matrix by large multinucleated osteoclasts is tightly coupled to the consequent formation of new bone by osteoblasts, such that lysis and formation are balanced (reviewed in Manolagas and Jilka, 1995). However, in prostate cancer bone metastasis, bone lysis is stimulated at sites of tumor growth and excess woven bone is synthesized [Clarke et al., 1991]. This results in a general increase in both bone turnover and volume, although woven bone has less collagen and therefore less tensile strength than normal and is more susceptible to fracture. Evidence suggests that lysis is a prerequisite for the establishment of tumor cells in bone [Roland, 1958; Nielsen et al., 1991], therefore understanding the regulation of bone resorption may suggest mechanisms through which tumors can develop in bone and may indicate novel therapeutic targets.

In normal bone, osteoblastic cells regulate osteoclastogenesis and osteoclast activity by interacting with mononuclear hematopoietic osteoclast precursors [Roodman, 1996]. The molecular mediators of this interaction were shown to be the osteoblast-expressed proteins, OPG and receptor activator of NFκB ligand (RANKL). Binding of RANKL to the osteoclast precursor-expressed RANK initiates a cascade of intracellular signals that culminates in the acquisition and activation of the osteoclast phenotype [Lacey et al., 1998; Yasuda et al., 1998a]. The absolute requirement of this interaction for osteoclastogenesis was shown by the generation of transgenic *rankl* −/− and *rank* −/− mice that developed severely hyperdense bones due to an absence of osteoclasts [Dougall et al., 1999; Kong et al., 1999]. Furthermore, administration of soluble extracellular RANKL to mice resulted in hypercalcemia and reduced bone volume, concomitant with a doubling of osteoclast size [Lacey et al., 1998]. The soluble glycoprotein OPG regulates excessive bone resorption by acting as a soluble decoy receptor for RANKL [Simonet et al., 1997], and therefore neutralizes its interaction with RANK, abrogating osteoclast formation, activation, and survival in vitro [Yasuda et al., 1998a,b] and in vivo [Lacey et al., 1998]. The crucial role of OPG in bone remodeling was demonstrated using transgenic *opg* −/− mice, which showed uncontrolled bone resorption and severe osteoporosis [Mizuno et al., 1998]. These studies suggest that the balance between RANKL and OPG determines the extent of bone resorption, in that a relative decrease in OPG results in excessive resorption and a relative increase in OPG inhibits resorption.

Recent work has shown that the expression of OPG, RANKL, and/or RANK is dysregulated in a number of cancers in bone, including osteoclastoma [Atkins et al., 2000] and prostate cancer [Brown et al., 2001], suggesting that these proteins may be involved in tumor-mediated bone destruction. Breast cancer cell lines were shown to express OPG and RANK but not RANKL [Thomas et al., 1999]. However, co-culture with hematopoietic bone marrow cells and osteoblasts resulted in a net increase in RANKL expression, suggesting an indirect mechanism through which localized bone lysis may occur in breast cancer bone metastasis, by activation of osteoclast precursors [Thomas et al., 1999]. This was supported using a murine in vitro model in which interactions between mouse breast cancer cells and bone marrow cells similarly resulted in a net increase in RANKL activity [Chikatsu et al., 2000]. The cancer–stromal interaction is also critical in multiple myeloma, where co-culture produced a net increase in RANKL expression and in osteoclastogenesis that was inhibited by addition of soluble RANK [Pearse et al., 2001]. The production of active soluble RANKL by prostate cancer cells in vitro has been implicated as a mechanism through which prostate cancer cells can directly initiate osteoclastogenesis and therefore stimulate bone resorption [Zhang et al., 2001].

Several exciting and provocative studies have examined the therapeutic uses of soluble RANK and OPG in the treatment of hematological and solid tumors in bone. As a fusion protein with human IgG, RANK has proven efficacious in the inhibition of bone resorption in a mouse model of humoral hypercalcemia of malignancy as induced by PTHrP administration [Oyajobi et al., 2001], and in the prevention of myeloma-induced osteoclastic bone destruction in a SCID-human model [Pearse et al., 2001]. In vitro experiments treating osteoclastoma-derived cells with OPG reduced the number of mature osteoclasts and inhibited bone resorption [Atkins et al., 2001]. Dramatic decreases in the numbers of mature osteoclasts and in the size and/or number of lesions in bone were observed following the treatment with OPG of mice carrying human breast cancer cells [Morony et al., 2001], murine multiple myeloma [Croucher et al., 2001], and human prostate cancer cells [Zhang et al., 2001]. In human prostate cancer cells, OPG has been shown to be a survival factor through its ability to inhibit TRAIL-mediated apoptosis [Holen et al., 2002]. Importantly, treatment with OPG has also been demonstrated to block pain-related behavior in mice carrying bone cancers [Honore et al., 2000; Luger et al., 2001]. Overall, these studies suggest that in bone metastatic tumors, inhibition of the primary resorptive stage may be sufficient to inhibit tumor establishment and halt progression of disease, even in those tumors that have primarily an osteoblastic phenotype. However, one prostate cancer cell line, LAPC-9, was demonstrated to not produce RANKL, but rather produced OPG [Lee et al., 2003]. This cell line produced osteoblastic tumor when injected into mouse tibia. The osteoblastic tumors did not appear to have osteoclastic activity during their early development phase, but developed osteoclastic activity by 6 weeks. These results bring into question the requirement for osteoclastic activity for the initial establishment of the prostate tumors in bone. Further support for this possibility was the observation that a bisphosphonate, zoledronic acid, did not diminish development of LAPC-9 cells injected into the tibia of mice; whereas it did decrease development of osteolytic PC-3 cells [Lee et al., 2002]. While studies are at an early stage at present, it appears that therapeutic targeting of the OPG/RANKL/RANK proteins holds great promise for at least therapy of bone metastases and perhaps may prevent establishment and progression of bone metastases.

A MODEL FOR PROSTATE CANCER'S EFFECT ON BONE REMODELING

From these observations, we propose a model for how prostate cancer cells influence bone remodeling. In order to account the apparently contrasting ability of prostate cancer cells to be both pro-osteoblastic and pro-osteolytic several aspects of the metastases need to be taken into account. These include the bone microenvironment the tumor cells are exposed to (reviewed in Cooper et al., 2003) and the temporal progression of the cancer. Based on these parameters, we propose (Fig. 1) that when prostate cancer cells metastasize to bone, they initially induce osteoclastogenesis and bone resorption. As bone is broken down, the extracellular matrix releases a variety of growth factors (reviewed in Guise and Mundy, 1998 #8470) that act in a paracrine fashion on the prostate tumor cells and diminish their ability to induce osteoclastogenesis, while promoting their ability to grow and induce osteoblastic activity. This model is consistent with various observations including the ability of anti-osteoclastogenic agents to inhibit establishment of tumor in bone and the mixture of osteolytic and osteoblastic features identified in clinical prostate cancer bone metastases, even within one patient. Unfortunately, proving this hypothesis is challenging for several reasons including that there are currently no animal models that recapitulate spontaneous clinical prostate cancer bone metastases.

The biology of prostate cancer bone metastasis has received increased attention in the last few years. The resulting data point to a complicated system with multiple interacting proteins and pathways. Thus, while dissecting individual protein factor pathways (e.g., BMPs) is important, eventually a synthesis of how these various pathways work together to impact bone remodeling will be necessary to provide a comprehensive understanding of the biology of prostate cancer bone metastases. Along this line of thought, clearly the bone microenvironment, which is under constant change from the influence of tumor cells, plays a role in the establishment and progression of prostate cancer bone metastases. Thus, future studies are needed to define the complex cross-talk between

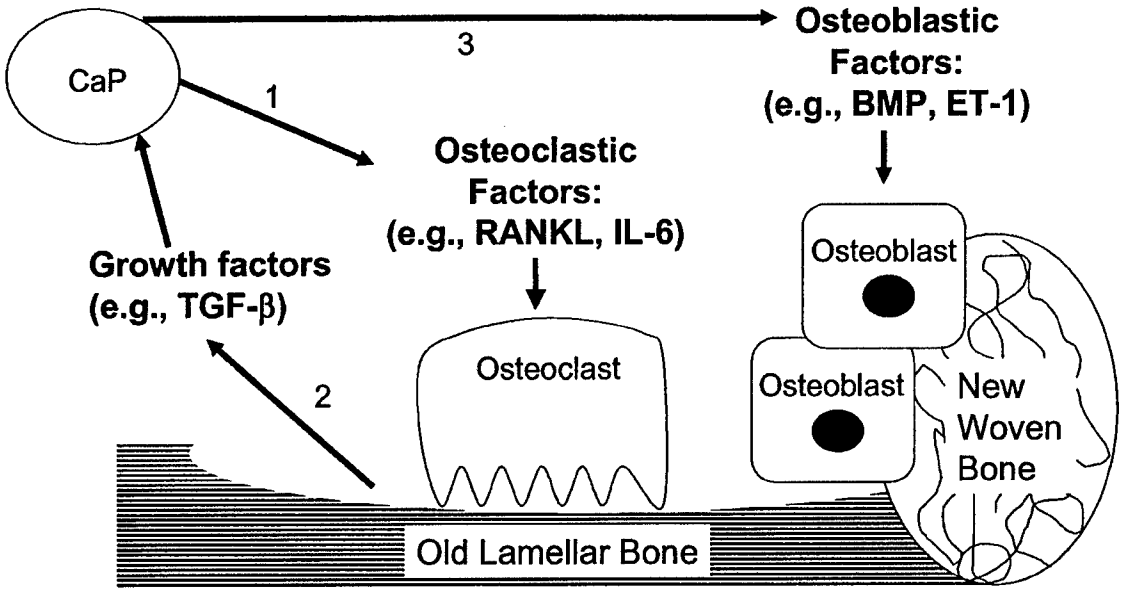

Fig. 1. Model for how prostate cancer induces bone remodeling. The prostate cancer cells initially (1) induce osteoclastogenesis and resorption of mature lamellar bone. As the bone matrix is destroyed, it releases growth factors (2) that induce prostate cancer cells' growth and alter their phenotype. The changing bone microenvironment, enhances the prostate cancer cells' production of osteoblastic factors (3) resulting in production of woven bone. BMP, bone morphogenetic protein; CaP, prostate cancer cell; ET-1, endothelin-1; IL-6, interleukin-6; RANKL, receptor activator of NFκB ligand; TGF-β, transforming growth factor β.

the bone microenvironment and the prostate cancer cells. In order to reach these goals, development of appropriate research tools, such as animal models and cells lines, that recapitulate human prostate cancer bone metastasis biology, are needed to advance the field.

REFERENCES

Abe E, Yamamoto M, Taguchi Y, Lecka-Czernik B, O'Brien CA, Economides AN, Stahl N, Jilka RL, Manolagas SC. 2000. Essential requirement of BMPs-2/4 for both osteoblast and osteoclast formation in murine bone marrow cultures from adult mice: Antagonism by noggin. J Bone Miner Res 15:663–673.

Abrams H, Spiro R, Goldstein N. 1950. Metastases in carcinoma. Cancer 3:74–85.

Achbarou A, Kaiser S, Tremblay G, Ste-Marie LG, Brodt P, Goltzman D, Rabbani SA. 1994. Urokinase overproduction results in increased skeletal metastasis by prostate cancer cells in vivo. Cancer Res 54:2372–2377.

Alam AS, Gallagher A, Shankar V, Ghatei MA, Datta HK, Huang CL, Moonga BS, Chambers TJ, Bloom SR, Zaidi M. 1992. Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: Implications for the vascular control of bone resorption. Endocrinology 130: 3617–3624.

Asahina I, Sampath TK, Nishimura I, Hauschka PV. 1993. Human osteogenic protein-1 induces both chondroblastic and osteoblastic differentiation of osteoprogenitor cells derived from newborn rat calvaria. J Cell Biol 123:921–933.

Atkins GJ, Haynes DR, Graves SE, Evdokiou A, Hay S, Bouralexis S, Findlay DM. 2000. Expression of osteoclast differentiation signals by stromal elements of giant cell tumors. J Bone Miner Res 15:640–649.

Atkins GJ, Bouralexis S, Haynes DR, Graves SE, Geary SM, Evdokiou A, Zannettino AC, Hay S, Findlay DM. 2001. Osteoprotegerin inhibits osteoclast formation and bone resorbing activity in giant cell tumors of bone. Bone 28:370–377.

Autzen P, Robson CN, Bjartell A, Malcolm AJ, Johnson MI, Neal DE, Hamdy FC. 1998. Bone morphogenetic protein 6 in skeletal metastases from prostate cancer and other common human malignancies. Br J Cancer 78:1219–1223.

Barnes J, Anthony CT, Wall N, Steiner MS. 1995. Bone morphogenetic protein-6 expression in normal and malignant prostate. World J Urol 13:337–343.

Bentley H, Hamdy FC, Hart KA, Seid JM, Williams JL, Johnstone D, Russell RG. 1992. Expression of bone morphogenetic proteins in human prostatic adenocarcinoma and benign prostatic hyperplasia. Br J Cancer 66:1159–1163.

Berruti A, Piovesan A, Torta M, Raucci CA, Gorzegno G, Paccotti P, Dogliotti L, Angeli A. 1996. Biochemical evaluation of bone turnover in cancer patients with bone metastases: Relationship with radiograph appearances and disease extension. Br J Cancer 73:1581–1587.

Blomme EA, Dougherty KM, Pienta KJ, Capen CC, Rosol TJ, McCauley LK. 1999. Skeletal metastasis of prostate adenocarcinoma in rats: Morphometric analysis and role of parathyroid hormone-related protein. Prostate 39: 187–197.

Boyce BF, Hughes DE, Wright KR, Xing L, Dai A. 1999a. Recent advances in bone biology provide insight into the pathogenesis of bone diseases. Lab Invest 79:83–94.

Boyce BF, Yoneda T, Guise TA. 1999b. Factors regulating the growth of metastatic cancer in bone. Endocr Relat Cancer 6:333–347.

Brown JM, Corey E, Lee ZD, True LD, Yun TJ, Tondravi M, Vessella RL. 2001. Osteoprotegerin and rank ligand expression in prostate cancer. Urology 57:611–616.

Brubaker KD, Vessella RL, Brown LG, Corey E. 2003. Prostate cancer expression of runt-domain transcription factor Runx2, a key regulator of osteoblast differentiation and function. Prostate 56:13–22.

Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC, Mihatsch MJ. 2000. Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. Hum Pathol 31:578–583.

Carlin BI, Andriole GL. 2000. The natural history, skeletal complications, and management of bone metastases in patients with prostate carcinoma. Cancer 88:2989–2994.

Charhon SA, Chapuy MC, Delvin EE, Valentin-Opran A, Edouard CM, Meunier PJ. 1983. Histomorphometric analysis of sclerotic bone metastases from prostatic carcinoma special reference to osteomalacia. Cancer 51:918–924.

Chiao JW, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, Ahmed T. 2000. Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. Br J Cancer 83:360–365.

Chikatsu N, Takeuchi Y, Tamura Y, Fukumoto S, Yano K, Tsuda E, Ogata E, Fujita T. 2000. Interactions between cancer and bone marrow cells induce osteoclast differentiation factor expression and osteoclast-like cell formation in vitro. Biochem Biophys Res Commun 267:632–637.

Clarke NW, McClure J, George NJ. 1991. Morphometric evidence for bone resorption and replacement in prostate cancer. Br J Urol 68:74–80.

Clarke NW, McClure J, George NJ. 1993. Osteoblast function and osteomalacia in metastatic prostate cancer. Eur Urol 24:286–290.

Coleman RE. 1997. Skeletal complications of malignancy. Cancer 80:1588–1594.

Cooper CR, Chay CH, Gendernalik JD, Lee HL, Bhatia J, Taichman RS, McCauley LK, Keller ET, Pienta KJ. 2003. Stromal factors involved in prostate carcinoma metastasis to bone. Cancer 97:739–747.

Cornish J, Callon KE, Lin C, Xiao C, Moseley JM, Reid IR. 1999. Stimulation of osteoblast proliferation by C-terminal fragments of parathyroid hormone-related protein. J Bone Miner Res 14:915–922.

Croucher PI, Shipman CM, Lippitt J, Perry M, Asosingh K, Hijzen A, Brabbs AC, van Beek EJ, Holen I, Skerry TM, Dunstan CR, Russell GR, Van Camp B, Vanderkerken K. 2001. Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. Blood 98:3534–3540.

Deftos LJ. 2000. Prostate carcinoma: Production of bioactive factors. Cancer 88:3002–3008.

Demers LM, Costa L, Lipton A. 2000. Biochemical markers and skeletal metastases. Cancer 88:2919–2926.

Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. 1999. RANK is essential for osteoclast and lymph node development. Genes Dev 13:2412–2424.

Ducy P, Karsenty G. 2000. The family of bone morphogenetic proteins. Kidney Int 57:2207–2214.

Ducy P, Schinke T, Karsenty G. 2000. The osteoblast: A sophisticated fibroblast under central surveillance. Science 289:1501–1504.

Fizazi K, Yang J, Peleg S, Sikes CR, Kreimann EL, Daliani D, Olive M, Raymond KA, Janus TJ, Logothetis CJ, Karsenty G, Navone NM. 2003. Prostate cancer cells–osteoblast interaction shifts expression of growth/survival-related genes in prostate cancer and reduces expression of osteoprotegerin in osteoblasts. Clin Cancer Res 9:2587–2597.

Galasko CS. 1986. Skeletal metastases. Clin Orthop 210: 18–30.

Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, Finegold MJ, Angelopoulou R, Rosen JM, Greenberg NM. 1996. Metastatic prostate cancer in a transgenic mouse. Cancer Res 56:4096–4102.

Goltzman D, Bolivar I, Rabbani SA. 1992. Studies on the pathogenesis of osteoblastic metastases by prostate cancer. Adv Exp Med Biol 324:165–171.

Goltzman D, Karaplis AC, Kremer R, Rabbani SA. 2000. Molecular basis of the spectrum of skeletal complications of neoplasia. Cancer 88:2903–2908.

Gori F, Thomas T, Hicok KC, Spelsberg TC, Riggs BL. 1999. Differentiation of human marrow stromal precursor cells: Bone morphogenetic protein-2 increases OSF2/CBFA1, enhances osteoblast commitment, and inhibits late adipocyte maturation. J Bone Miner Res 14:1522–1535.

Groeneveld EH, Burger EH. 2000. Bone morphogenetic proteins in human bone regeneration. Eur J Endocrinol 142:9–21.

Guise TA. 2000. Molecular mechanisms of osteolytic bone metastases. Cancer 88:2892–2898.

Guise TA, Mundy GR. 1998. Cancer and bone. Endocr Rev 19:18–54.

Hall BK, Miyake T. 2000. All for one and one for all: Condensations and the initiation of skeletal development. Bioessays 22:138–147.

Hamdy FC, Autzen P, Robinson MC, Horne CH, Neal DE, Robson CN. 1997. Immunolocalization and messenger RNA expression of bone morphogenetic protein-6 in human benign and malignant prostatic tissue. Cancer Res 57:4427–4431.

Harris SE, Harris MA, Mahy P, Wozney J, Feng JQ, Mundy GR. 1994. Expression of bone morphogenetic protein messenger RNAs by normal rat and human prostate and prostate cancer cells. Prostate 24:204–211.

Hogan BL. 1996. Bone morphogenetic proteins in development. Curr Opin Genet Dev 6:432–438.

Holen I, Croucher PI, Hamdy FC, Eaton CL. 2002. Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells. Cancer Res 62:1619–1623.

Honore P, Luger NM, Sabino MA, Schwei MJ, Rogers SD, Mach DB, O'Keefe PF, Ramnaraine ML, Clohisy DR, Mantyh PW. 2000. Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain, and pain-related neurochemical reorganization of the spinal cord. Nat Med 6:521–528.

Hullinger TG, Taichman RS, Linseman DA, Somerman MJ. 2000. Secretory products from PC-3 and MCF-7 tumor cell lines upregulate osteopontin in MC3T3-E1 cells. J Cell Biochem 78:607–616.

Ide H, Katoh M, Sasaki H, Yoshida T, Aoki K, Nawa Y, Osada Y, Sugimura T, Terada M. 1997a. Cloning of human bone morphogenetic protein type IB receptor (BMPR-IB) and its expression in prostate cancer in comparison with other BMPRs published erratum appears in Oncogene 1997 Aug 28;15(9):1121. Oncogene 14:1377–1382.

Ide H, Yoshida T, Matsumoto N, Aoki K, Osada Y, Sugimura T, Terada M. 1997b. Growth regulation of human prostate cancer cells by bone morphogenetic protein-2. Cancer Res 57:5022–5027.

Jena N, Martin-Seisdedos C, McCue P, Croce CM. 1997. BMP7 null mutation in mice: Developmental defects in skeleton, kidney, and eye. Exp Cell Res 230:28–37.

Karaplis AC, Vautour L. 1997. Parathyroid hormone-related peptide and the parathyroid hormone/parathyroid hormone-related peptide receptor in skeletal development. Curr Opin Nephrol Hypertens 6:308–313.

Karsenty G. 2000. Bone formation and factors affecting this process. Matrix Biol 19:85–89.

Katagiri T, Yamaguchi A, Ikeda T, Yoshiki S, Wozney JM, Rosen V, Wang EA, Tanaka H, Omura S, Suda T. 1990. The non-osteogenic mouse pluripotent cell line, C3H10T1/2, is induced to differentiate into osteoblastic cells by recombinant human bone morphogenetic protein-2. Biochem Biophys Res Commun 172:295–299.

Katagiri T, Akiyama S, Namiki M, Komaki M, Yamaguchi A, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T. 1997. Bone morphogenetic protein-2 inhibits terminal differentiation of myogenic cells by suppressing the transcriptional activity of MyoD and myogenin. Exp Cell Res 230:342–351.

Killian CS, Corral DA, Kawinski E, Constantine RI. 1993. Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-beta and a proteolytic modulation of cell adhesion receptors. Biochem Biophys Res Commun 192:940–947.

Kim KJ, Itoh T, Kotake S. 1997. Effects of recombinant human bone morphogenetic protein-2 on human bone marrow cells cultured with various biomaterials. J Biomed Mater Res 35:279–285.

Kim IY, Lee DH, Ahn HJ, Tokunaga H, Song W, Devereaux LM, Jin D, Sampath TK, Morton RA. 2000. Expression of bone morphogenetic protein receptors type-IA, -IB, and -II correlates with tumor grade in human prostate cancer tissues. Cancer Res 60:2840–2844.

Kimura G, Sugisaki Y, Masugi Y, Nakazawa N. 1992. Calcification in human osteoblasts cultured in medium conditioned by the prostatic cancer cell line PC-3 and prostatic acid phosphatase. Urol Int 48:25–30.

Kingsley DM, Bland AE, Grubber JM, Marker PC, Russell LB, Copeland NG, Jenkins NA. 1992. The mouse short ear skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGF beta superfamily. Cell 71:399–410.

Knutsen R, Wergedal JE, Sampath TK, Baylink DJ, Mohan S. 1993. Osteogenic protein-1 stimulates proliferation and differentiation of human bone cells in vitro. Biochem Biophys Res Commun 194:1352–1358.

Kong YY, Boyle WJ, Penninger JM. 1999. Osteoprotegerin ligand: A common link between osteoclastogenesis, lymph node formation, and lymphocyte development. Immunol Cell Biol 77:188–193.

La M, Reid JJ. 1995. Endothelin-1 and the regulation of vascular tone. Clin Exp Pharmacol Physiol 22:315–323.

Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176.

Laitinen M, Marttinen A, Aho AJ, Lindholm TS. 1998. Bone morphogenetic protein in bone neoplasms: Comparison of different detection methods. Eur Surg Res 30:168–174.

Lange PH, Vessella RL. 1998. Mechanisms, hypotheses, and questions regarding prostate cancer micrometastases to bone. Cancer Metastasis Rev 17:331–336.

Le Brun G, Aubin P, Soliman H, Ropiquet F, Villette JM, Berthon P, Creminon C, Cussenot O, Fiet J. 1999. Upregulation of endothelin 1 and its precursor by IL-1beta, TNF-alpha, and TGF-beta in the PC3 human prostate cancer cell line. Cytokine 11:157–162.

Lee YP, Schwarz EM, Davies M, Jo M, Gates J, Zhang X, Wu J, Lieberman JR. 2002. Use of zoledronate to treat osteoblastic versus osteolytic lesions in a severe-combined-immunodeficient mouse model. Cancer Res 62: 5564–5570.

Lee Y, Schwarz E, Davies M, Jo M, Gates J, Wu J, Zhang X, Lieberman JR. 2003. Differences in the cytokine profiles associated with prostate cancer cell induced osteoblastic and osteolytic lesions in bone. J Orthop Res 21: 62–72.

Li IW, Cheifetz S, McCulloch CA, Sampath KT, Sodek J. 1996. Effects of osteogenic protein-1 (OP-1, BMP-7) on bone matrix protein expression by fetal rat calvarial cells are differentiation stage specific. J Cell Physiol 169: 115–125.

Lin DL, Tarnowski CP, Zhang J, Dai J, Rohn E, Patel AH, Morris MD, Keller ET. 2001. Bone metastatic LNCaP-derivative C4-2B prostate cancer cell line mineralizes in vitro. Prostate 47:212–221.

Luger NM, Honore P, Sabino MA, Schwei MJ, Rogers SD, Mach DB, Clohisy DR, Mantyh PW. 2001. Osteoprotegerin diminishes advanced bone cancer pain. Cancer Res 61:4038–4047.

Maeda H, Koizumi M, Yoshimura K, Yamauchi T, Kawai T, Ogata E. 1997. Correlation between bone metabolic markers and bone scan in prostatic cancer. J Urol 157:539–543.

Maliakal JC, Asahina I, Hauschka PV, Sampath TK. 1994. Osteogenic protein-1 (BMP-7) inhibits cell proliferation and stimulates the expression of markers characteristic of osteoblast phenotype in rat osteosarcoma (17/2.8) cells. Growth Factors 11:227–234.

Manolagas SC, Jilka RL. 1995. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. New Engl J Med 332:305–311.

Masuda H, Fukabori Y, Nakano K, Takezawa Y, T CS, Yamanaka H. 2003. Increased expression of bone morphogenetic protein-7 in bone metastatic prostate cancer. Prostate 54:268–274.

Merino R, Rodriguez-Leon J, Macias D, Ganan Y, Economides AN, Hurle JM. 1999. The BMP antagonist gremlin regulates outgrowth, chondrogenesis, and programmed cell death in the developing limb. Development 126:5515–5522.

Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H. 1998. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun 247:610–615.

Morony S, Capparelli C, Sarosi I, Lacey DL, Dunstan CR, Kostenuik PJ. 2001. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. Cancer Res 61:4432–4436.

Moul JW, Lipo DR. 1999. Prostate cancer in the late 1990s: Hormone refractory disease options. Urol Nurs 19:125–31; quiz 132-3.

Munk PL, Poon PY, O'Connell JX, Janzen D, Coupland D, Kwong JS, Gelmon K, Worsley D. 1997. Osteoblastic metastases from breast carcinoma with false-negative bone scan. Skeletal Radiol 26:434–437.

Nelson JB, Carducci MA. 2000. The role of endothelin-1 and endothelin receptor antagonists in prostate cancer. BJU Int 85(Suppl 2):45–48.

Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW. 1995. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat Med 1:944–949.

Nelson JB, Chan-Tack K, Hedican SP, Magnuson SR, Opgenorth TJ, Bova GS, Simons JW. 1996. Endothelin-1 production and decreased endothelin B receptor expression in advanced prostate cancer. Cancer Res 56:663–668.

Nelson JB, Nguyen SH, Wu-Wong JR, Opgenorth TJ, Dixon DB, Chung LW, Inoue N. 1999. New bone formation in an osteoblastic tumor model is increased by endothelin-1 overexpression and decreased by endothelin A receptor blockade. Urology 53:1063–1069.

Nielsen OS, Munro AJ, Tannock IF. 1991. Bone metastases: Pathophysiology and management policy. J Clin Oncol 9:509–524.

Nifuji A, Noda M. 1999. Coordinated expression of noggin and bone morphogenetic proteins (BMPs) during early skeletogenesis and induction of noggin expression by BMP-7. J Bone Miner Res 14:2057–2066.

Nifuji A, Kellermann O, Noda M. 1999. Noggin expression in a mesodermal pluripotent cell line C1 and its regulation by BMP. J Cell Biochem 73:437–444.

Oyajobi BO, Anderson DM, Traianedes K, Williams PJ, Yoneda T, Mundy GR. 2001. Therapeutic efficacy of a soluble receptor activator of nuclear factor kappaB–IgG Fc fusion protein in suppressing bone resorption and hypercalcemia in a model of humoral hypercalcemia of malignancy. Cancer Res 61:2572–2578.

Papapoulos SE, Hamdy NA, van der Pluijm G. 2000. Bisphosphonates in the management of prostate carcinoma metastatic to the skeleton. Cancer 88:3047–3053.

Parfitt AM. 2000. The mechanism of coupling: A role for the vasculature. Bone 26:319–323.

Patel KV, Schrey MP. 1995. Human breast cancer cells contain a phosphoramidon-sensitive metalloproteinase which can process exogenous big endothelin-1 to endothelin-1: A proposed mitogen for human breast fibroblasts. Br J Cancer 71:442–447.

Patel KV, Sheth HG, Schrey MP. 1997. Stimulation or endothelin-1 secretion by human breast cancer cells through protein kinase A activation: A possible novel paracrine loop involving breast fibroblast-derived prostaglandin E2. Mol Cell Endocrinol 126:143–151.

Pearse RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF, Colman N, Michaeli J, Epstein J, Choi Y. 2001. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci USA 98:11581–11586.

Percival RC, Urwin GH, Harris S, Yates AJ, Williams JL, Beneton M, Kanis JA. 1987. Biochemical and histological evidence that carcinoma of the prostate is associated with increased bone resorption. Eur J Surg Oncol 13:41–49.

Perkel VS, Mohan S, Baylink DJ, Linkhart TA. 1990. An inhibitory insulin-like growth factor binding protein (In-IGFBP) from human prostatic cell conditioned medium reveals N-terminal sequence identity with bone derived In-IGFBP. J Clin Endocrinol Metab 71:533–535.

Pirtskhalaishvili G, Nelson JB. 2000. Endothelium-derived factors as paracrine mediators of prostate cancer progression. Prostate 44:77–87.

Rabbani SA, Gladu J, Mazar AP, Henkin J, Goltzman D. 1997. Induction in human osteoblastic cells (SaOS2) of the early response genes *fos*, *jun*, and *myc* by the amino terminal fragment (ATF) of urokinase. J Cell Physiol 172:137–145.

Rana A, Chisholm GD, Khan M, Sekharjit SS, Merrick MV, Elton RA. 1993. Patterns of bone metastasis and their prognostic significance in patients with carcinoma of the prostate. Br J Urol 72:933–936.

Raval P, Hsu HH, Schneider DJ, Sarras MP, Jr., Masuhara K, Bonewald LF, Anderson HC. 1996. Expression of bone morphogenetic proteins by osteoinductive and non-osteoinductive human osteosarcoma cells. J Dent Res 75:1518–1523.

Reddi AH. 1997. Bone morphogenetic proteins: An unconventional approach to isolation of first mammalian morphogens. Cytokine Growth Factor Rev 8:11–20.

Roland S. 1958. Calcium studies in ten cases of osteoblastic prostatic metastasis. J Urol 79:339–342.

Roodman GD. 1996. Advances in bone biology: The osteoclast. Endocr Rev 17:308–332.

Rosol TJ. 2000. Pathogenesis of bone metastases: Role of tumor-related proteins. J Bone Miner Res 15:844–850.

Roudier M, Sherrard D, True L, Ott-Ralp S, Meligro C, MBerrie M, Soo C, Felise D, Quinn JE, Vessella R. 2000. Heterogenous bone histomorphometric patterns in metastatic prostate cancer. J Bone Miner Res 15S1:S567.

Sasaki T, Hong MH. 1993a. Endothelin-1 localization in bone cells and vascular endothelial cells in rat bone marrow. Anat Rec 237:332–337.

Sasaki T, Hong MH. 1993b. Localization of endothelin-1 in the osteoclast. J Electron Microsc (Tokyo) 42:193–196.

Schrey MP, Patel KV. 1995. Prostaglandin E2 production and metabolism in human breast cancer cells and breast fibroblasts. Regulation by inflammatory mediators. Br J Cancer 72:1412–1419.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL,

Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Boyle WJ, et al. 1997. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89:309–319.

Solloway MJ, Dudley AT, Bikoff EK, Lyons KM, Hogan BL, Robertson EJ. 1998. Mice lacking Bmp6 function. Dev Genet 22:321–339.

Stern PH, Tatrai A, Semler DE, Lee SK, Lakatos P, Strieleman PJ, Tarjan G, Sanders JL. 1995. Endothelin receptors, second messengers, and actions in bone. J Nutr 125:2028S–2032S.

Suzuki A, Kaneko E, Maeda J, Ueno N. 1997. Mesoderm induction by BMP-4 and -7 heterodimers. Biochem Biophys Res Commun 232:153–156.

Szostak MJ, Kyprianou N. 2000. Radiation-induced apoptosis: Predictive and therapeutic significance in radiotherapy of prostate cancer (review). Oncol Rep 7:699–706.

Taguchi Y, Yamamoto M, Yamate T, Lin SC, Mocharla H, DeTogni P, Nakayama N, Boyce BF, Abe E, Manolagas SC. 1998. Interleukin-6-type cytokines stimulate mesenchymal progenitor differentiation toward the osteoblastic lineage. Proc Assoc Am Physicians 110: 559–574.

Takuwa Y, Masaki T, Yamashita K. 1990. The effects of the endothelin family peptides on cultured osteoblastic cells from rat calvariae. Biochem Biophys Res Commun 170:998–1005.

Thalmann GN, Anezinis PE, Chang S-M, Zhau HE, Kim EE, Hopwood VL, Pathak S, Eschenbach ACv, Chung LWK. 1994. Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. Cancer Res 54:2577–2581.

Thomas RJ, Guise TA, Yin JJ, Elliott J, Horwood NJ, Martin TJ, Gillespie MT. 1999. Breast cancer cells interact with osteoblasts to support osteoclast formation. Endocrinology 140:4451–448.

Tsuji K, Ito Y, Noda M. 1998. Expression of the *PEBP2alphaA/AML3/CBFA1* gene is regulated by BMP4/7 heterodimer and its overexpression suppresses type I collagen and osteocalcin gene expression in osteoblastic and nonosteoblastic mesenchymal cells. Bone 22:87–92.

Urwin GH, Percival RC, Harris S, Beneton MN, Williams JL, Kanis JA. 1985. Generalised increase in bone resorption in carcinoma of the prostate. Br J Urol 57: 721–723.

Vinholes J, Coleman R, Eastell R. 1996. Effects of bone metastases on bone metabolism: Implications for diagnosis, imaging, and assessment of response to cancer treatment. Cancer Treat Rev 22:289–331.

Wan M, Shi X, Feng X, Cao X. 2001. Transcriptional mechanisms of BMP-induced osteoprotegrin gene expression. J Biol Chem 276:10119–10125.

Weber KL, Bolander ME, Rock MG, Pritchard D, Sarkar G. 1998. Evidence for the upregulation of osteogenic protein-1 mRNA expression in musculoskeletal neoplasms. J Orthop Res 16:8–14.

Wlosarski K, Reddi AH. 1987. Tumor cells stimulate in vivo periosteal bone formation. Bone Miner 2:185–192.

Wozney JM. 1992. The bone morphogenetic protein family and osteogenesis. Mol Reprod Dev 32:160–167.

Wrana JL. 2000. Regulation of Smad activity. Cell 100:189–192.

Yamaguchi A, Ishizuya T, Kintou N, Wada Y, Katagiri T, Wozney JM, Rosen V, Yoshiki S. 1996. Effects of BMP-2, BMP-4, and BMP-6 on osteoblastic differentiation of bone marrow-derived stromal cell lines, ST2, and MC3T3-G2/PA6. Biochem Biophys Res Commun 220:366–371.

Yamashita T, Yoshitake H, Tsuji K, Kawaguchi N, Nabeshima Y, Noda M. 2000. Retardation in bone resorption after bone marrow ablation in klotho mutant mice. Endocrinology 141:438–445.

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415.

Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. 1998a. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): A mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology 139:1329–1337.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. 1998b. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602.

Yin J, Grubbs B, Cui Y, Weu-Wong J, Wessale J, Padley R, Guise T. 2000. Endothelin A receptor blockade inhibits osteoblastic metastases. J Bone Miner Res 15:S201.

Yoneda T. 1998. Cellular and molecular mechanisms of breast and prostate cancer metastasis to bone. Eur J Cancer 34:240–245.

Yorimitsu K, Moroi K, Inagaki N, Saito T, Masuda Y, Masaki T, Seino S, Kimura S. 1995. Cloning and sequencing of a human endothelin converting enzyme in renal adenocarcinoma (ACHN) cells producing endothelin-2. Biochem Biophys Res Commun 208:721–727.

Zhang J, Dai J, Qi Y, Lin DL, Smith P, Strayhorn C, Mizokami A, Fu Z, Westman J, Keller ET. 2001. Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. J Clin Invest 107:1235–1244.

Zimmerman LB, De Jesus-Escobar JM, Harland RM. 1996. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell 86:599–606.